

jc498 U.S. PTO
04/19/99

Practitioner's Docket No. 48235

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

jc612 U.S. PTO
09/29/98
04/19/99

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

Inventor(s):

Richard L. HUGANIR and Jee Hae KIM

WARNING: 37 C.F.R. § 1.41(a)(1) points out

"(a) A patent is applied for in the name or names of the actual inventor or inventors

"(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title):

SIGNAL TRANSDUCING SYNAPTIC MOLECULES AND USES THEREOF

CERTIFICATION UNDER 37 C.F.R. 1.10*

(Express Mail label number is **mandatory**.)

(Express Mail certification is optional.)

I hereby certify that this New Application Transmittal and the documents referred to as attached therein are being deposited with the United States Postal Service on this date **April 19, 1999** in an envelope as "Express Mail Post Office to Addressee," mailing Label Number **EI978002370US**, addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

Peter F. Corless

(type or print name of person mailing paper)

Signature of person mailing paper

WARNING: Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing 37 C.F.R. 1.10(b).

"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.

(Application Transmittal [4-1]—page 1 of 11)

1. Type of Application

This new application is for a(n)

(check one applicable item below)

- ☒ Original (nonprovisional)
☐ Design
☐ Plant

WARNING: Do not use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.

WARNING: Do not use this transmittal for the filing of a provisional application.

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

- ☐ Divisional.
☒ Continuation.
☐ Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

WARNING: When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application **must** be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).

- ☒ The new application being transmitted claims the benefit of prior U.S. application(s).

3. Papers Enclosed

- A. Required for filing date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

68 Pages of specification

11 Pages of claims

23 Sheets of drawing

WARNING: **DO NOT** submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 CFR 1.84, see Notice of March 9, 1988 (1990 O.G. 57-62).

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page." 37 C.F.R. 1.84(c)).

(complete the following, if applicable)

- ☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. 1.84(b).
- ☐ formal
- ☐ informal

B. Other Papers Enclosed

- ____ Pages of declaration and power of attorney
- ____ Pages of abstract
- ____ Other

4. Additional papers enclosed

- ☐ Amendment to claims
- ☐ Cancel in this applications claims _____ before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
- ☐ Add the claims shown on the attached amendment. (Claims added have been numbered consecutively following the highest numbered original claims.)
- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement (37 C.F.R. 1.98)
- ☐ Form PTO-1449 (PTO/SB/08A and 08B)
- ☐ Citations

- ☐ Declaration of Biological Deposit
- ☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
- ☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative
- ☐ Special Comments
- ☐ Other

5. Declaration or oath (including power of attorney)

NOTE: A newly executed declaration is not required in a continuation or divisional application provided that the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47, then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. §§ 1.63(d)(1)–(3).

NOTE: A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name including family name and at least one given name, without abbreviation together with any other given name or initial, and the residence, post office address and country or citizenship of each inventor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. § 1.63(a)(1)–(4).

- ☐ Enclosed

Executed by

(check all applicable boxes)

- ☐ inventor(s).
- ☐ legal representative of inventor(s).
37 CFR 1.42 or 1.43.
- ☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

- ☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. See item 13 below for fee.

- ☒ Not Enclosed.

NOTE: Where the filing is a completion in the U.S. of an International Application or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

- ☒ Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of all the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently).

- ☐ Showing that the filing is authorized.
(not required unless called into question. 37 CFR 1.41(d))

6. Inventorship Statement

WARNING: If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.

The inventorship for all the claims in this application are:

☐ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of \$130.00 required by 37 CFR 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 CFR 1.52(d).

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. 1.52(d).

8. Assignment

☐ An assignment of the invention to _____

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☐ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters—one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: A newly executed "CERTIFICATE UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.

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9. Certified Copy

Certified copy(ies) of application(s)

Country	Appln. No.	Filed
Country	Appln. No.	Filed
Country	Appln. No.	Filed

from which priority is claimed

☐ is (are) attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. 1.16)

A. ☐ Regular application

CLAIMS AS FILED			
Number filed	Number Extra	Rate	Basic Fee 37 C.F.R. 1.16(a) \$790.00
Total			
Claims (37 CFR 1.16(c))	- 20 =	×	\$ 22.00
Independent			
Claims (37 CFR 1.16(b))	- 3 =	×	\$ 82.00
Multiple dependent claim(s), if any (37 CFR 1.16(d))		+	\$270.00

☐ Amendment cancelling extra claims is enclosed.

☐ Amendment deleting multiple-dependencies is enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation \$_____

B. ☐ Design application
(\$330.00—37 CFR 1.16(f))

Filing Fee Calculation \$_____

C. ☐ Plant application
(\$540.00—37 CFR 1.16(g))

Filing fee calculation \$_____

11. Small Entity Statement(s)

- ☐ Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached.

WARNING: "Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).

(complete the following, if applicable)

- ☒ Status as a small entity was claimed in prior application
60 / 082717, filed on April 23, 1999, from which benefit
is being claimed for this application under:

35 U.S.C. ☒ 119(e),
☐ 120,
☐ 121,
☐ 365(c),

and which status as a small entity is still proper and desired.

- ☒ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of A, B or C above)

\$ _____

NOTE: Any excess of the full fee paid will be refunded if small entity status is established and a refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 CFR 1.28(a).

12. Request for International-Type Search (37 C.F.R. 1.104(d))

(complete, if applicable)

- ☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

(Application Transmittal [4-1]—page 7 of 11)

13. Fee Payment Being Made at This Time

☒ Not Enclosed

☒ No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. 1.16(e) can be paid subsequently.)

☐ Enclosed

☐ Filing fee \$ _____

☐ Recording assignment
(\$40.00; 37 C.F.R. 1.21(h))
(See attached "COVER SHEET FOR
ASSIGNMENT ACCOMPANYING NEW
APPLICATION".) \$ _____

☐ Petition fee for filing by other than all the
inventors or person on behalf of the inventor
where inventor refused to sign or cannot be
reached
(\$130.00; 37 C.F.R. 1.47 and 1.17(i)) \$ _____

☐ For processing an application with a
specification in
a non-English language
(\$130.00; 37 C.F.R. 1.52(d) and 1.17(k)) \$ _____

☐ Processing and retention fee
(\$130.00; 37 C.F.R. 1.53(d) and 1.21(l)) \$ _____

☐ Fee for international-type search report
(\$40.00; 37 C.F.R. 1.21(e)) \$ _____

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 CFR 1.53(f) and this, as well as the changes to 37 CFR 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total fees enclosed \$ _____

14. Method of Payment of Fees

☐ Check in the amount of \$ _____

☐ Charge Account No. _____ in the amount of
\$ _____

A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: If no fees are to be paid on filing, the following items should not be completed.

WARNING: Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.

- ☐ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. _____:

- ☐ 37 C.F.R. 1.16(a), (f) or (g) (filing fees)
☐ 37 C.F.R. 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

- ☐ 37 C.F.R. 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)
☐ 37 C.F.R. §§ 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a)).
☐ 37 C.F.R. 1.17 (application processing fees)

NOTE: “. . . A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission.” 37 C.F.R. § 1.136(a)(3).

- ☐ 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires “Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . the issue fee. . . .” From the wording of 37 CFR 1.28(b), (a) notification of change of status must be made even if the fee is paid as “other than a small entity” and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

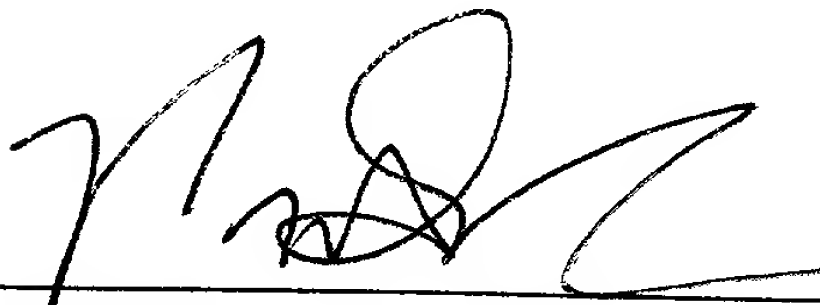
NOTE: "... Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

- ☐ Credit Account No. _____
- ☐ Refund

Reg. No. 33860

Tel. No. (617) 523-3400

Customer No.



SIGNATURE OF PRACTITIONER

Peter F. Corless

(type or print name of attorney)

Dike, Bronstein, Roberts & Cushman LLP

P.O. Address

130 Water Street
Boston, MA 02109

☐ **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

- ☐ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added _____

- ☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added _____

- ☐ Plus added pages deleting names of inventor(s) named in prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added _____

- ☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added _____

☐ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

- ☒ This transmittal ends with this page.

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY
STATUS (37 CFR 1.9(f) AND 1.27 (d)) - NONPROFIT ORGANIZATION**

Docket No.
48235-P1

Serial No.

60/082,717

Filing Date

April 23, 1998

Patent No.

Issue Date

Applicant/ **Richard L. HUGANIR and Jee Hae KIM**
Patentee:

Invention: **SIGNAL TRANSDUCING SYNAPTIC MOLECULES AND USES THEREOF**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: Johns Hopkins University

ADDRESS OF ORGANIZATION: 720 Rutland Avenue
Baltimore, Maryland 21205
TYPE OF NONPROFIT ORGANIZATION:

- ☒ University or other Institute of Higher Education
- ☐ Tax Exempt under Internal Revenue Service Code (26 U.S.C. 501(a) and 501(c)(3))
- ☐ Nonprofit Scientific or Educational under Statute of State of The United States of America
Name of State: Citation of Statute:
- ☐ Would Qualify as Tax Exempt under Internal Revenue Service Code (26 U.S.C. 501(a) and 501(c)(3)) if Located in The United States of America
- ☐ Would Qualify as Nonprofit Scientific or Educational under Statute of State of The United States of America if Located in The United States of America
Name of State: Citation of Statute:

I hereby declare that the above-identified nonprofit organization qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in:

- ☐ the specification to be filed herewith.
- ☒ the application identified above.
- ☐ the patent identified above.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the above-identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☒ no such person, concern or organization exists.
☐ each such person, concern or organization is listed below.

FULL NAME _____
 ADDRESS _____

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____
 ADDRESS _____

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____
 ADDRESS _____

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____
 ADDRESS _____

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

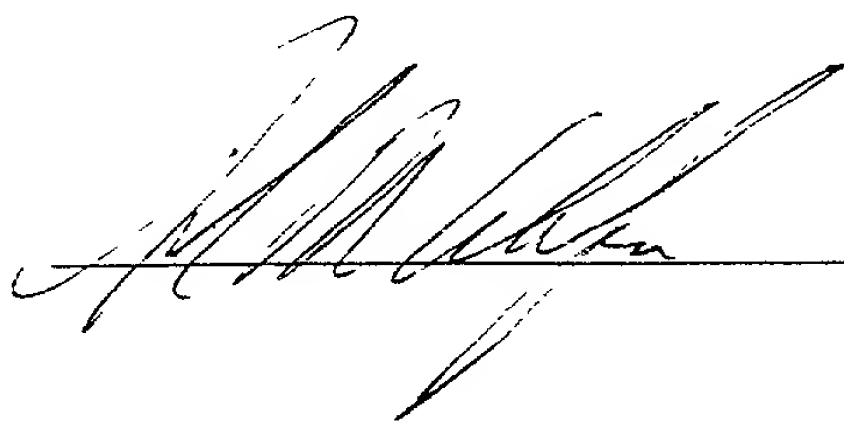
Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Howard W. Califano
 TITLE IN ORGANIZATION: Assistant Dean and Director
 ADDRESS OF PERSON SIGNING: 2024 East Monument Street, Suite 2-100
Baltimore, Maryland 21205

SIGNATURE: _____



DATE: _____

6-1-98

SIGNAL TRANSDUCING SYNAPTIC MOLECULES AND USES THEREOF

BACKGROUND OF THE INVENTION

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of pending U.S. provisional application serial number 60/082,690 filed on April 22, 1998, and pending U.S. provisional application serial number 60/082,717 filed on April 23, 1998, the disclosures of both provisional applications fully incorporated herein by reference.

1. Field of the Invention

The present invention relates to signal transducing synaptic molecules and particularly to mammalian SYNGAP (Synaptic GTPase Activating Protein), including
5 recombinant SYNGAPs and fragments and derivatives thereof. In one aspect, the invention provides molecules for detecting and analyzing SYNGAPs *in vitro* and *in vivo*. In another aspect, the invention provides assays for detecting compounds that modulate SYNGAP or SYNGAP-related activities. The invention has a variety of applications including use in screens to detect pharmacological agents useful in the diagnosis or
10 treatment of disorders associated with SYNGAP.

2. Background

Neurons communicate by a variety of means including synaptic transmission. One form of synaptic transmission involves chemical signaling; a process generally
15 involving neurotransmitter release from one neuron and modulation of a post-synaptic receptor in another neuron. The release and modulation is usually manifested by propagation of a chemical or electrical impulse. See Edelman, G.M. et al. (eds.) (1987) in *Synaptic Function*, New York: Wiley; and Goodman and Gilman (1996) in *The Pharmacological Basis of Therapeutics*, 9th ed. J.G. Hardman, et al. (eds) Pergamon
20 Press, NY.

In many instances, chemical signaling requires specialized neuronal structures called chemical synapses. Analysis of chemical synapses has attracted substantial interest. For example, chemical synapses have been reported to be involved in many, if not all, nervous system functions including neuronal plasticity. In particular, neuronal plasticity is believed to impact critical functions such as cognition, e.g., memory and learning, as well as certain neurodegenerative disorders. See e.g., Kandel, E.R. et al; (1991) in *Principles of Neuroscience*, Appleton & Lange, Norwalk, CT and references cited therein.

A variety of approaches have been used in attempts to understand chemical synapses. For example, certain molecular and biochemical approaches have suggested that chemical synapses are structured and include molecules such as receptors, cytoskeletal proteins, and signal transduction molecules. See e.g., Ehlers, M.D., et al. (1996) *Curr. Opin. Cell Biol.* 8: 484; Sheng, M. (1996) *Neuron* 17:575; and Huganir, R.L. and Greengard, M. (1990) *Neuron* 5: 555.

More particularly, it has been reported that appropriate chemical synapse structure requires presence of a protein termed PSD-95/SAP90. The PSD-95/SAP90 protein is representative of a family of molecules including SAP120. Specific members of the PSD-95/SAP90 family are localized at or near chemical synapses. See e.g., Ehlers, M.D., et al. *supra*; Sheng, M *supra*; Lau, L. F., et al. (1996) *J. Biol. Chem.* 271:21622; Muller, B.M., et al. (1996) *Neuron* 17:255; and references cited therein.

There has much effort towards understanding PSD-95/SAP90 and related proteins. For example, it has been reported that most members of the protein family exhibit the same or a closely related structure, i.e., three tandem PDZ (PSD-95, DLG, ZO-1) domains, a SH3 (src homology 3) domain, and an inactive yeast guanylate kinase domain (GK). See e.g., Kim, E., et al. (1995) *Nature* 378:85; Kornau, H. C., et al. (1995) *Science* 269:1737.

There has been recognition that PDZ domains exist in proteins associated with cell membranes. For example, it has been reported that the PDZ domains of PSD-95 bind to specific subunits of the (N-methyl-D-aspartate) NMDA receptor. It has also been reported that the last three amino acids of the NMDA receptor subunits define a consensus sequence (T/SXV; X is any amino acid). The subunit sequence has been reported to facilitate the binding. See e.g., Kennedy, M. B. (1995) *Trends Biochem. Sci.* 20; Gomperts, S. N. (1996) *Cell* 84:659; Sheng, M. *supra*.

There has also been recognition that chemical synapses can employ signal transduction to modulate pre- and post-synaptic activity. For example, certain members of the PSD-95/SAP90 protein family are believed to be associated with signal transduction. More particularly, the PSD-95 protein has been proposed to interact with certain signal transduction kinases. That interaction has been proposed to be important to synaptic function. See Gomperts, *supra*; Saras, J., and Heldin, C. H. (1996) *Trends Biochem. Sci.* 21:455; Huganir, R.L. and Greengard, M. *supra*.

There has also been recognition that the PSD-95/SAP90 protein may be capable of serving as an adaptor molecule. More specifically, there have been reports that the PSD-95/SAP90 protein may be able to relate synaptic activity and signal transduction in some instances. That property is believed to important to appropriate synapse function. See e.g., Brenman, J.E. et al. (1996) *Cell* 84: 757; and Saras, J. and Heldin, Carl-Henrik (1996), *supra*.

A variety of signal transduction molecules are known including specified kinases and proto-oncogenes. For example, the proto-oncogene Ras is recognized as a G-protein that is apparently involved in signal transduction pathways affecting, e.g., cell growth, cell differentiation, neuronal plasticity and cell survival. In particular, Ras appears to have a substantial role in kinase activation. In addition, biological activity manifested by a variety of neurotrophic factors (i.e. neurotrophins) may be derived through Ras-

associated signaling pathways. See e.g., Bokoch, G. M., and Der, C. J. (1993) *FASEB. J.* 7:750; Marshall, C. J. (1996) *Cell Biol.* 8:197; Finkbeiner, S. and Greenberg, M.E. (1996) *Neuron* 16:233; Kang, H., and Schuman, E. M. (1996) *Science* 273:1402.

5 Certain neuronal functions have been proposed to be affected by Ras-mediated signal transduction. See e.g., Seger, R., and Krebs, E. G. (1995) *FASEB J.* 9:726; and Finkbeiner and Greenberg, *supra*.

10 Additional signal transduction pathways are known. For example, the inositol triphosphate signaling pathway has been reported to couple modulation of certain receptors to a variety of functions, many of which relate to calcium. See e.g., Berridge, M.J. (1988) *Pro. R. Soc. Lond. (Biol.)* 234: 359.

15 It would be desirable to identify molecules that impact chemical synapse function and particularly interact with the PSD-95/SAP90 protein. It would be further desirable to identify molecules that can bind the PSD-95/SAP90 protein and affect signal transduction. It would also be desirable to have effective assays for identifying compounds and especially pharmaceutical agents with capacity to modulate the function of these molecules.

20 SUMMARY OF THE INVENTION

25 The present invention features molecules that relate to SYNGAP (Synaptic GTPase Activating Protein); an excitatory synapse protein that has been found to bind synaptic proteins and modulate signal transduction. In one aspect, the invention provides isolated polynucleotides that encode SYNGAP or fragments or derivatives thereof. Further provided are SYNGAP or SYNGAP-related polypeptide encoded by the polynucleotides. In another aspect, the invention provides immunological molecules that are capable of binding the polypeptides. Additionally provided are methods for using the molecules of this invention, e.g., to treat or prevent at least one disorder mediated by

SYNGAP. The invention also provides screening assays for detecting compounds useful in the diagnosis or treatment of disorders impacted by SYNGAP.

We have discovered mammalian SYNGAP: a novel protein that binds synaptic proteins important for chemical synapse function. Additionally, we have found that SYNGAP is capable of modulating certain signal transduction molecules and particularly the Ras proto-oncogene (Ras). We have particularly found that SYNGAP is positioned to relate synaptic activity and signal transduction, thereby indicating a significant role in many aspects of nervous system function.

As will be discussed below, SYNGAP is represented by a family of alternatively spliced variants including SYNGAP-a, SYNGAP-b, and SYNGAP-c.

The present invention provides a number of significant uses and advantages. For example, the invention relates, for the first time, recognized synaptic proteins such as PSD95/SAP90 and SAP120 to signal transduction. More particularly, the invention provides SYNGAP and SYNGAP-related molecules that are believed to serve as adaptors between key synaptic proteins and specific transduction pathways. Accordingly, the present invention is expected to facilitate attempts to more fully understand relations between synapses and signal transduction, particularly relationships between synaptic function and signaling molecules such as Ras, inositol triphosphate and certain other transduction molecules.

The present invention has a wide spectrum of important applications. For example, specific molecules of this invention can be used as a diagnostic tool to detect excitatory synapses, i.e., those that include mammalian SYNGAP. Illustrative of such excitatory synapses are those impacting neuronal plasticity, particularly habituation, sensitization, learning and memory; as well as certain neurological disorders. In addition, the invention can be employed in the diagnosis, treatment or prophylaxis of certain neurological disorders impacted by SYNGAP. As will be discussed in more detail below,

exemplary neurological conditions include those specifically affecting awareness and cognition as well as neuronal growth and survival.

Further, the present invention provides a variety of highly useful molecular markers including polynucleotides, polypeptides, and immune system molecules that can be used in many commercial, medical, home or research settings. For example, certain molecules of this invention can be used in screening assays to detect compounds and particularly pharmaceutical agents useful in the diagnosis or treatment of neurological disorders impacted by SYNGAP. Of particular interest are recognized manipulations that can be employed for the identification of small molecules, e.g., synthetic peptides, peptide mimetics, drugs, etc., that can modulate interaction between SYNGAP and the synaptic proteins to which it associates; interaction between SYNGAP and signal transduction molecules or both. Related techniques can be used to screen for small molecules that potentially block or enhance one or all of these interactions. In particular, *in vitro* screens are provided that can detect SYNGAP antagonists or agonists.

Additional uses include use of SYNGAP polynucleotides to detect SYNGAP expression in desired cells or groups of cells such as tissue or an organ by conventional *in situ* hybridization methods.

Particularly useful are SYNGAP-binding immune system molecules of this invention such as the antibodies and antigen-binding antibody fragments provided below. For example, the antibodies (monoclonal and polyclonal) can be used alone or in combination with other agents to facilitate identification of excitatory chemical synapses that include SYNGAP. In some instances, it may be desirable to include the antibodies wholly or as part of a therapeutic strategy to monitor or in some cases modulate the excitatory chemical synapses. Preferred molecules of the invention are flexible and can be modified, if desired, to deliver a desired molecule such as a drug, toxin, enzyme or radionuclide optionally through a linker sequence such as a peptide linker sequence. As

an illustration, a SYNGAP binding antibody of this invention can be detectably-labeled to identify excitatory chemical synapses *in vitro* or *in vivo*.

The SYNGAP and SYNGAP-related molecules of this invention have additional
5 uses and advantages *in vitro* and *in vivo*. For example, the molecules can be employed in functional, cellular and molecular assays (e.g., screens) and in structural analysis, including X-ray crystallography, nuclear magnetic resonance imaging (NMRI), computational techniques. Of particular interest are those techniques involving computer-assisted simulation of synapses (i.e. formulation of virtual synapses). Also
10 included are useful techniques for using the SYNGAP and SYNGAP-related molecules as markers that can provide diagnostic imaging of excitatory chemical synapses *in vivo*. By way of illustration, certain molecules of this invention can be employed to visualize specific neurons and particularly chemical synapses including SYNGAP in the brain of a living patient. In this embodiment, the desired molecule will usually be detectably-
15 labeled with a suitable tag such as a radionuclide or other suitable imaging component known in the field.

Specific SYNGAP and SYNGAP-related molecules of this invention can be provided in a kit form or other convenient form to facilitate manufacture, packaging,
20 dissemination, storage, and/or use of the present invention.

Accordingly, in one aspect, the invention provides isolated polynucleotides (RNA, mRNA, cDNA, genomic DNA, or chimeras thereof) that encode SYNGAP or a fragment or derivative of SYNGAP. Illustrative of such polynucleotides include those
25 encoding a mammalian SYNGAP, e.g., a primate and particularly a rat or a human SYNGAP. In one embodiment, the polynucleotide encodes a mammalian SYNGAP having a molecular weight of between about 100 to about 150 kDa or greater. In another embodiment, the polynucleotide has at least about 70 percent sequence identity to any of the nucleotide sequences shown in SEQ ID NOS. 1-2, 4-5, or 7-8. Such sequence
30 similarity (i.e. about 70%) or greater similarity will sometimes be referred to herein as

"substantial homology" or like term. Specifically preferred polynucleotides of the invention encode the rat SYNGAP shown in any of the SEQ ID Nos. 3, 6, or 9.

In another aspect, the present invention provides an isolated polynucleotide that is capable of hybridizing to the nucleotide sequence shown in SEQ ID Nos. 1-2, 4-5, or 7-8 under moderate stringency hybridization conditions. In a preferred embodiment, the polynucleotide will also hybridize to those specific sequences shown in SEQ ID NOS. 1-2, 4-5, or 7-8 under high stringency conditions. The terms "moderate" and "high" hybridization stringency have readily understandable meaning to those of skill in this field. Exemplary stringency conditions are disclosed in the discussion and examples which follow. In one embodiment, the polynucleotide capable of hybridizing to the SEQ ID NO: 1 or SEQ ID NO: 2 under high stringency conditions is between from about 12 to about 50 nucleotides in length. Illustrative of such polynucleotides are oligonucleotide primers made by conventional synthetic methods. In another embodiment, the polynucleotide is between about 100 to about 3500 nucleotides in length or greater. Illustrative of such polynucleotides are restriction enzyme fragments or chemically synthesized fragments complementary to the sequences shown in SEQ ID Nos. 1-2, 4-5, or 7-8.

In another embodiment of the invention, the polynucleotide capable of hybridizing to the nucleotide sequence shown in SEQ ID Nos. 1-2, 4-5, or 7-8 under high stringency conditions has a length of between about 100 up to about 4000 nucleotides or greater. In a preferred embodiment, the polynucleotide is a cDNA encoding an amino acid sequence capable of modulating the proto-oncogene Ras as determined, e.g., by a standard Ras GTPase activity assay. In a specific embodiment, the amino acid sequence encoded by the polynucleotide is capable inhibiting the Ras protein by at least about 10% up to about 100% in the standard Ras GTPase assay. Additionally preferred are polynucleotides that encode polypeptides capable of modulating Ras-mediated signaling transduction pathways and optionally signal transduction molecules "downstream" of Ras (directly or indirectly). Examples of such downstream molecules include recognized

signaling and effector molecules. Illustrative methods for identifying such polynucleotides and amino acid sequences are described below.

One or a combination of standard approaches can be used to monitor Ras GTPase activity in cells or cell lysates. Preferably, the cell or cell lysate will include a naturally-occurring or recombinant Ras protein. Preferred assays for measuring and quantitating the Ras GTPase activity are discussed below.

Additionally preferred polynucleotides of this invention encode a mammalian SYNGAP or fragment or derivative thereof that is capable of significantly reducing inositol triphosphate signaling as determined, e.g., by modulation of phospholipase C activity in a standard phospholipase C enzyme assay. Preferably, the polynucleotide is a cDNA that is capable of increasing or decreasing the enzyme activity by at least about 10% or more up to about 100% relative to a suitable control assay. Specific phospholipase C assays are described below.

Additionally preferred polynucleotides of this invention encode a mammalian SYNGAP or a SYNGAP-related amino acid sequence that is capable of binding at least from about 1, 2 or 3 up to about 10 PDZ domains as determined by a standard PDZ domain binding assay. As noted above, the PDZ domain has been reported to be present in a variety of membrane proteins, e.g., the PSD95/SAP90 and SAP120 proteins, and is believed to significantly impact chemical synapses.

By the term "SYNGAP-related" nucleotide or amino acid sequence or similar term is meant a fragment or derivative of SYNGAP sequence (polynucleotide or polypeptide as described below).

A variety of methods are known in this field for detecting and quantifying, if desired, PDZ binding. In general, the methods are capable of detecting formation of a binding complex and can be optimized to provide qualitative or quantitative

characterization of those binding complexes. Illustrative methods for detecting the PDZ binding are more fully disclosed in the discussion and examples that follow.

5 Additionally preferred polynucleotides are those molecules that encode an amino acid sequence that includes at least a Ras GTPase Activating Protein (GAP) domain up to about 2 to about 3 GAP domains, and a C-terminal sequence that includes at least the following general amino acid sequence: (T or S), X V; wherein X is an amino acid, preferably one of the 20 natural amino acids.

10 Further preferred are those polynucleotides that encode an amino acid sequence that includes at least one pleckstrin homology (PH) up to about 3 PH domains and at least one C2 domain up to about 3 C2 domains.

15 Particularly preferred polynucleotides of this invention encode an amino acid sequence that includes in an N- to C-terminal orientation: at least one PH homology, preferably one PH homology; at least one C2 domain, preferably one C2 domain; at least one GAP domain, preferably one GAP domain; and the C-terminal sequence having the sequence (T or S), X V described above. Additionally preferred is a polynucleotide encoding an amino acid sequence that includes in an N- to C-terminal direction at least
20 about the following amino acids of SEQ ID NO. 9: 4 to 72, 87 to 190, 266 to 502 and 1132 to 1135.

Specifically preferred are those polynucleotides that encode a rat SYNGAP as represented by any one of SEQ ID NOS. 3, 6 or 8 including fragments or derivatives
25 thereof.

One or a combination of different strategies can be used to analyze the SYNGAP or SYNGAP-related molecules disclosed herein, e.g., to detect homologous molecules or to identify protein domains (e.g., GAP, PH, and C2). Specifically included are

biochemical, immunological and biosensor-type assays as well as certain well-known computer-assisted manipulations.

Additionally provided by the present invention are fragments or derivatives of the polynucleotides encoding mammalian SYNGAP or SYNGAP-related molecules, as well as recombinant vectors including the polynucleotides or the fragments or derivatives thereof. It is generally preferred that the recombinant vector be capable of propagating the isolated polynucleotide in a suitable prokaryotic or eukaryotic host cell. Additionally preferred recombinant vectors are capable of expressing that isolated polynucleotide as RNA and preferably mRNA, in a suitable cell expression system. The recombinant vector can include nearly any number of useful elements, however in most cases the vector will include control elements operably linked to the inserted nucleic acid (e.g., promoter, leader, and/or enhancer elements) which control elements can be selected to optimize replication and/or transcription of the vector in the cells.

As noted, polynucleotides of the invention generally encode mammalian SYNGAP or a fragment or derivative thereof. In one embodiment, the polynucleotides are substantially homologous to the SYNGAP sequence shown in SEQ ID NO: 3, 6 or 9. In a specific embodiment, the isolated polynucleotides include or consists of cDNA and have a length of between about 50 to about 100 nucleotides up to about 4000 nucleotides or more, as determined by standard nucleic acid sizing methods. In another embodiment, the isolated nucleic acid includes or consists of RNA and particularly mRNA that is also substantially homologous to the specific rat SYNGAP sequence and which can have substantially the same length as the cDNA.

Also provided are host cells that include a polynucleotide as disclosed herein including those that express SYNGAP, a SYNGAP-related molecule or a fragment or derivative thereof under suitable cell culture conditions. Preferably, the host cells are capable of expressing the desired amino acid sequence in the host cell, cell medium, or both.

The invention also includes methods for isolating a polynucleotide encoding a mammalian SYNGAP or SYNGAP-related molecule such as the rat SYNGAP sequence specifically described below. In general, the methods include introducing the polynucleotide into host cells, typically as a recombinant vector including the polynucleotide, culturing the host cells under conditions suitable for propagating the polynucleotide and purifying the polynucleotide from the host cells to obtain larger portion of the isolated polynucleotide therefrom. Host cells useful for propagating the polynucleotides and/or polypeptides of this invention can be eukaryotic, prokaryotic, more particularly, fungal, yeast, animal or insect as desired. Host cells amenable for large scale production of mammalian SYNGAP are especially useful for commercial and industrial applications. Alternatively PCR amplification or related amplification methods can be used to isolate the polynucleotide.

Further provided are cultured host cells which have been transformed, transfected or infected either transiently or stably by at least one recombinant vector of the invention which vector includes an isolated polynucleotide that encodes a mammalian SYNGAP or a fragment or derivative thereof (DNA or RNA).

Recombinant vectors of the invention can be introduced into suitable cells or groups of such cells including tissue or organs if desired either *in vitro* or *in vivo*. Preferably, the cells are capable of expressing the recombinant vector at detectable levels. Host cells comprising the vectors can be cultured in medium capable of supporting propagation and/or expression of the vectors in the cells. The cells can be eukaryotic cells, preferably mammalian cells such as neurons and neuron-associated cells (e.g., glia) which cells are capable of expressing desired sequences in the recombinant vector. The cells can be primary cells or the cells can be immortalized. In some instances it will be desirable to introduce the vector into a suitable prokaryotic host e.g., bacteria, insect, yeast or fungal cells to propagate the vector.

The present invention also provides useful oligonucleotide primers, typically single-stranded primers, which oligonucleotide primers are complementary to a polynucleotide encoding a mammalian SYNGAP. For example, in one embodiment, the oligonucleotide primers are complementary to the rat SYNGAP sequence shown in any one of SEQ ID Nos. 1-2, 4-5, or 7-8. The oligonucleotide primers have a variety of useful applications, e.g., to detect or amplify a mammalian SYNGAP of interest. Exemplary oligonucleotide primers will generally have length of between about 12 to about 70 nucleotides although somewhat larger or smaller primers are useful for some applications.

Additional polynucleotides of the present invention have important uses. For example, as discussed, the invention provides for recombinant vectors that include an isolated polynucleotide relating to SYNGAP. Specific recombinant vectors can be used to produce significant amounts of nucleic acid sequence that can be sense or anti-sense, single-stranded or double-stranded as needed. Generally, RNA transcribed from DNA is referred to as the "sense" RNA strand and oppositely oriented RNA is termed antisense RNA. Antisense polynucleotides, then, refer to sequences of DNA or RNA which can bind in a Watson-Crick fashion to a sequence on a target mRNA. See generally Bentley, D. L. and Groudine, M. (1986) *Nature* 321:702; and Kimelman, D. *Gene regulation: Biology of Antisense RNA and DNA*, R. P. Erickson, J. G. Izant, eds. (Raven Press, New York).

Mammalian SYNGAP RNA and particularly a SYNGAP mRNA existing in a biological sample such as in an excitatory chemical synapse (*in vivo* or *in vitro*) will sometimes be referred to herein as a "target" to denote potential for specific binding between a polynucleotide of interest, e.g., a suitable anti-sense RNA, and the SYNGAP mRNA in the sample.

In one preferred embodiment, the recombinant vectors include DNA sequences that encode an anti-sense RNA which RNA is substantially homologous to mammalian

SYNGAP polynucleotides of this invention e.g., a rat SYNGAP. It is preferred that the recombinant vectors include cDNA sequences. In this instance it will be understood that the anti-sense RNA will usually include a uracil (U) in place of thymidine (T) where the cDNA sequence has a thymidine. In a preferred embodiment, the anti-sense RNA has a
5 length of at least about 20 to about 50 nucleotides, at least about 100 to about 250 nucleotides, at least about 300 to about 700 nucleotides, at least about 1000 to about 2000 nucleotides and up to about 2500 to about 4000 nucleotides as determined by standard polynucleotide sizing methods. In most cases, the length of the anti-sense RNA will be guided by intended use including the length of the target and the level of anti-sense
10 suppression desired.

The antisense RNA encoded by specific recombinant vectors of this invention is usually designed to undergo complementary base pairing (hybridization) with the target, rendering the target essentially unavailable for translation in most cases. In some
15 instances, the antisense RNA will render the target susceptible to degradation, thereby substantially reducing the amount of the target in relevant cells or tissue. Accordingly, the recombinant vectors of the invention can be used to control the synthesis and/or expression of SYNGAP in desired neurons *in vitro* or *in vivo*.

Specific recombinant vectors of this invention that are capable of producing anti-sense RNA complementary to a mammalian SYNGAP mRNA (or more than one of such mRNA) can be used therapeutically to reduce levels of the target *in vivo* or *in vitro*. For example, in one embodiment, a desired recombinant vector is administered to a patient suffering from or suspected of suffering from a SYNGAP related disorder such as those
20 specified herein. In this instance, the patient will benefit from substantially reduced or totally absent levels of the target. Preferred administration is sufficient to reduce levels of the target mRNA in the patient. Efficacy of the technique can be monitored and quantified if desired by a variety of techniques including Northern and Western blotting.

In another embodiment, the recombinant vector is formatted to produce anti-sense DNA of about the same size as the anti-sense RNA.

In addition, the polynucleotides of this invention and particularly the isolated nucleic acids and recombinant vectors described herein can be used as important controls for detecting and analyzing normal and aberrant mammalian SYNGAP expression *in vitro* and *in vivo*.

In another aspect, the present invention provides isolated mammalian SYNGAP preferably having an apparent molecular weight of between about 100 to about 150 kDa. The molecular weight of the SYNGAP can be determined by a variety of standard means including polyacrylamide gel electrophoresis. Preferred are polypeptide sequences having at least about 200 amino acids up to about 1000 amino acids or greater including about 1100 amino acids. Specifically provided is rat SYNGAP as shown at the amino acid sequence level in any one of SEQ ID NOS. 3, 6 or 9.

In another aspect, there is provided an isolated polypeptide having at least about 70 percent amino acid sequence homology to the sequence illustrated in SEQ ID NO: 3, 6 or 9. Additionally provided are isolated fragments or derivatives of that polypeptide.

The invention also provides methods for producing a mammalian SYNGAP in which the method includes culturing the host cell in medium under conditions suitable for expression of the SYNGAP in the host cell or medium.

In another aspect, the present invention provides an antibody or antigen-binding fragment thereof capable of binding the amino acid sequence shown in SEQ ID NO. 9. Preferably, the binding of the antibody or antigen-binding fragment is blocked by at least about 80%, 90% or more up to about 100% by contact with the amino acid sequence shown in SEQ ID NO:21 or a sequence substantially homologous thereto. The percent

blocking by the amino acid sequence can be determined by a variety of means including a conventional immunoprecipitation assay or a Western immunoblot.

In one preferred embodiment, the antibody is capable of binding excitatory
5 synapses as determined by microscopy. In this embodiment, the antibody can be a monoclonal or a polyclonal antibody or an antigen-binding fragment thereof. In most cases, the antibody or antigen-binding fragment will be detectably-labeled or will be capable of generating a detectable label as specified below to help visualize and optionally quantitate synaptic binding.

10 Additionally, the invention pertains to methods for making specific immune system molecules and particularly antibodies (polyclonal, monoclonal or chimeric molecules) which bind certain SYNGAP or a SYNGAP-related molecules described herein. The methods generally include using a substantially purified sample of a desired
15 SYNGAP polypeptide, as immunogen. Exemplarily antibodies are monoclonal antibodies obtained by conventional hybridoma manipulations. The antibodies can also be generated from an immunogenic peptide that comprises one or more epitopes of the polypeptide. It will be useful in some settings to covalently attach a suitable cytotoxic, anti-metabolic, or detectable label to the antibody by methods well-known in the field to
20 help detect and/or modulate desired synapses that include mammalian SYNGAP and particularly human or rat SYNGAP *in vitro* or *in vivo*.

It will be understood that an "immune system molecule" generally relates to antibodies and antigen-binding fragments of those antibodies that are derived from the
25 immune system of a mammal such as a mouse, rat, rabbit, human, and the like.

The immune system molecules of this invention provide a number of uses and advantages. For example, the immune system molecules can be used to modulate mammalian SYNGAP expression *in vitro* or *in vivo*, e.g., by employing conventional
30 microinjection techniques. In one approach, a suitable amount of a desired SYNGAP

antibody is suspended in a physiologically acceptable buffer and injected into a desired cell or group of cells including tissue or an organ, which amount is sufficient to reduce or eliminate SYNGAP expression as determined, e.g., by the biochemical and functional assays described herein. In some instances, such approaches will be well-suited to modulate SYNGAP function, e.g., to impact neuronal plasticity *in vitro* or *in vivo*.

In another aspect, the present invention provides a kit that typically includes at least one container means comprising at least one of: 1) an antibody or antigen-binding fragment thereof capable of binding mammalian SYNGAP, 2) an isolated polynucleotide comprising sequence with at least about 70% sequence homology to any one of the sequences shown in SEQ ID NOs: 1-2, 4-5, or 7-8; 3) a pair of oligonucleotide primers capable of hybridizing to any one of the sequences shown in SEQ ID NOs: 1-2, 4-5, or 7-8, preferably under high stringency conditions; and 4) a polypeptide with at least about 70% to about 100% sequence homology to the sequence shown in any one of the sequences shown in SEQ ID NOs: 3, 6 or 9 or a fragment or a derivative thereof.

In one embodiment, the kit includes a system for: 1) treating or preventing a disorder in a mammal associated with the SYNGAP, 2) detecting excitatory synapses in a cell or group of cells *in vitro* or *in vivo*, or both.

Isolated polynucleotides and polypeptides of the invention can be obtained as a substantially pure preparation if desired. That is, the nucleic acids and polypeptides can be isolated in substantially pure form by standard methods and can be provided as sterile preparations if desired. Methods for providing substantially pure preparations of nucleic acids and polypeptides are discussed below.

In another aspect of the present invention, there is provided methods for modulating excitatory synapse function in a cell or group of cells *in vitro* or *in vivo*. In this embodiment, the method includes administering to the cells a modulation effective amount of at least one polynucleotide of this invention or fragment or derivative thereof.

By the term “modulation effective” is meant a change in exciting synapse function (e.g., amount of SYNGAP).

The present invention also includes methods for modulating excitatory synapse function in a cell or group of cells *in vitro* or *in vivo*. In this embodiment, the method includes administering to the cells a modulation effective amount of a mammalian SYNGAP of this invention, including a fragment or derivative thereof.

In embodiments of the methods for modulating excitatory synapse function, the modulation can include an increase in excitatory synapse number. Excitatory synapses can be visualized and quantified, if desired, by a variety of means including microscopy or centrifugation.

In another aspect, the present invention includes methods for treating a disorder associated with a mammalian SYNGAP including administering to a patient suffering from or susceptible to such disorder an effective amount of at least one isolated polynucleotide of this invention or fragment or derivative thereof.

Additionally provided are methods for treating a disorder associated with a mammalian SYNGAP comprising administering to a patient suffering from or susceptible to such disorder an effective amount of an isolated SYNGAP of this invention or fragment or derivative thereof.

In embodiments of the methods for treating SYNGAP-related disorders, the disorder can be a neurological disorder of the central (CNS) or peripheral (PNS) nervous system. In a specific embodiment, the CNS disorder is at least one of an affective disorder, a cognitive disorder, or a neurodegenerative disorder. For example, the affective disorder can be depression; the cognitive disorder can be at least one of memory loss, a learning disability, or schizophrenia; and the learning disability can be attention deficit disorder (ADD). In another specific embodiment, the degenerative disorder can be

at least one of Parkinson's disease (PD), Huntington's disease (HD), senile dementia, or Alzheimer's disease (AD). In a further specific embodiment, the PNS disorder is amyotrophic lateral sclerosis. In another specific embodiment, the neurological disorder is associated with at least one of trauma, an immune response or ischemia.

5

The methods for treating or preventing the SYNGAP-related disorders will also find applicability in the manipulation of habituation, sensitization, learning and memory in research settings.

10

As noted, the present invention provides a variety of screening assays for detecting compounds and especially pharmaceutical agents for use in the diagnosis or treatment of disorders impacted by mammalian SYNGAP.

15

In one embodiment, there is provided methods for identifying a compound useful in the diagnosis or treatment of a disorder relating to the SYNGAP. In a specific embodiment, the method includes at least one of the following steps:

20

- a) culturing cells capable of forming synapses comprising SYNGAP under conditions conducive to forming or maintaining synapses,
- b) contacting the cells with a candidate compound,
- c) analyzing the cells for an increase or decrease in the number of synapses; and
- d) detecting the increase or decrease as indicative of the compound useful in the diagnosis or treatment of the disorder relating to SYNGAP.

25

In another embodiment, the invention provides methods for detecting a compound capable of modulating a Ras-activated second messenger pathway. In this embodiment, the method includes at least one of the following steps:

- a) providing a Ras response system comprising a recombinant mammalian GTPase Activating Protein at Synapses (SYNGAP),
- b) contacting the Ras response system with a candidate compound,

- c) analyzing the Ras response system for an increase or decrease in Ras activity;
- and
- d) detecting the increase or decrease in the Ras activity as indicative of the compound capable of modulating the Ras-activated second messenger pathway.

5

In preferred embodiments, the Ras response system which includes at least one of Ras a nucleotide di- or tri-phosphate; adenylate cyclase; and an isolated polynucleotide encoding the SYNGAP or a fragment or a derivative thereof. The Ras response system can be provided by one or a combination different strategies including being provided in a host cell or a group of host cells (e.g., tissue or an organ), or a lysate of the cells, tissue or organ.

In another embodiment, the invention includes methods for detecting a compound capable of modulating a Ras-Raf (MAP kinase) cascade. The RAS-GTPase activating activity of SYNGAP can be measured using the MAP kinase as a reporter in a suitable primary or cultured cells. In this embodiment, the method includes at least one of the following steps:

- a) transfecting a cells with a reporter gene construct capable of being modulated by Ras-Raf (MAP kinase),
- b) transfecting the cells with a polynucleotide encoding any one of the SYNGAP sequences shown in SEQ ID Nos. 3, 6, or 9,
- c) contacting the cells with a candidate compound; and
- d) detecting the reporter gene construct as being indicative of the compound capable of modulating the RasGTPase activity of SYNGAP.

25

It will be appreciated that in some embodiments of the method, the SYNGAP cDNA can be provided before, during or after transfection of the reporter gene construct.

In another embodiment, the invention includes methods for detecting a compound capable of modulating a phospholipid-activated second messenger pathway. In this embodiment, the method includes at least one of the following steps:

- a) providing an inositol triphosphate response system comprising a recombinant SYNGAP,
- b) contacting the inositol triphosphate response system with a candidate compound,
- c) analyzing the inositol triphosphate response system for an increase or decrease in phospholipase activity; and
- d) detecting the increase or decrease in the phospholipase activity as indicative of the compound capable of modulating the phospholipid-activated second messenger pathway. In preferred embodiments, the phospholipase is a phosphoinositide-specific enzyme such as phospholipase C.

In one embodiment of the method, the inositol triphosphate response system further includes at least one of phosphatidylinositol (PI) and an isolated polynucleotide encoding the SYNGAP or a fragment or a derivative thereof. In a specific embodiment, the inositol triphosphate response system includes at least one of diacylglycerol, protein kinase C, and inositol 1,4,5 triphosphate (INSP₃). In a related embodiment, the phospholipid-activated second messenger pathway is capable of at least one of calcium (Ca²⁺) release or protein phosphorylation. The inositol triphosphate response system can be provided by one or a combination different strategies including being provided in a host cell or a group of host cells (e.g., tissue or an organ), or a lysate of the cells, tissue or organ.

In another embodiment of the method, at least the pleckstrin (PH) homology of SYNGAP is provided for use with the inositol triphosphate response system. In another embodiment of the method, at least the PH homology and the SYNGAP C2 domain is used with the inositol triphosphate response system.

Additionally provided by the present invention are methods for detecting a compound capable of modulating phospholipid-dependent calcium (Ca^{+2}) binding to at least the SYNGAP C2 domain. In this embodiment, the method includes at least one of the following steps:

- a) mixing a phospholipid, calcium (Ca^{+2}) and at least the SYNGAP C2 domain, the mixing being under conditions conducive to forming a complex,
- b) contacting the mixture with a candidate compound,
- c) analyzing the mixture for formation of the complex; and
- d) detecting the complex as indicative of the compound capable of modulating the phospholipid-dependent binding between the calcium (Ca^{+2}) and at least the C2 domain of SYNGAP.

In another embodiment of the method, at least the PH homology and the SYNGAP C2 domain is used with the inositol triphosphate response system.

In embodiments of the present invention in which use of rat SYNGAP is desired, it will be possible to use a polypeptide sequence that includes and preferably consists of the sequence represented by any one of SEQ ID Nos : 3, 6 or 9. Also contemplated are embodiments of the present invention which include use of a fragment or a derivative of the rat SYNGAP sequence as show in SEQ ID NOs: 3, 6 or 9.

The methods for detecting the compound capable of modulating the phospholipid-activated second messenger pathway are flexible and can be used if one or more components of the inositol triphosphate response system, e.g., PI, act “upstream” or “downstream” of SYNGAP.

In another embodiment of the present invention, there is provided methods for detecting a test amino acid sequence capable of binding a mammalian SYNGAP. In one embodiment, the method includes contacting the test amino acid sequence with the mammalian SYNGAP or a binding fragment or the derivative thereof under conditions

conducive to forming a complex; and detecting the complex as indicative of the test amino acid sequence capable of binding the SYNGAP. For example, in a specific embodiment, the mammalian SYNGAP can be the rat SYNGAP sequence shown in SEQ ID NO: 9 or a suitable fragment or derivative thereof. Detection of the binding can be accomplished by one or a combination of different strategies such as at least one of immunoprecipitation, affinity chromatography, or a suitable biosensor assay.

In another embodiment of the method for detecting a test amino acid sequence capable of binding a mammalian SYNGAP, the contacting step can be performed in cells and the detecting step can include monitoring expression of a detectable gene product expressed by the cells. In a preferred embodiment, the steps are performed in yeast cells. Particularly preferred are those yeast cells (strains) that are suited for performing what is generally known in the field as a "two-hybrid" assay or a related term. In other embodiments, the detecting step of the methods can include screening a polypeptide expression library or a combinatorial peptide library, e.g., by a hybridization type assay using a suitable polynucleotide of this invention, for the test amino acid sequence.

In preferred instances, the methods for detecting the binding between the test amino acid sequence and the SYNGAP will typically register binding between at least one PDZ domain in the test amino acid sequence and a C-terminal sequence in the SYNGAP or fragment or derivative thereof. Preferably, the C-terminal sequence has the following general sequence: (T or S) XV; wherein X is an amino acid as defined below. The SYNGAP sequence used can be the rat SYNGAP sequence disclosed in SEQ ID NO: 3, 6 or 9 as well as suitable fragments or derivatives of that sequence.

Additionally provided are amino acid sequences detected by any of the methods for detecting the test amino acid sequence capable of binding a mammalian SYNGAP.

The present invention includes, in another aspect, methods for detecting excitatory synapses in a cell or group of cells. In one embodiment, the methods include contacting

the cells or group of cells with an antibody or antigen-binding fragment of this invention under conditions sufficient to detect the excitatory synapses in the cells or group of cells. In a specific embodiment, the antibody or antigen-binding fragment is detectably-labeled or is capable of producing a detectable label. Illustrative of such labels include a
5 radionuclide; a protein tag; a chromophore; a fluorescent, chemiluminescent or phosphorescent molecule. In another specific embodiment the antibody or antigen-binding fragment thereof is labeled with at least one enzyme capable of producing a chromophore, a fluorescent, chemiluminescent or phosphorescent molecule.

10 In another aspect, the present invention provides a library that includes a plurality of the polynucleotides or the polypeptides of this invention including fragments or derivatives of those polynucleotides or polypeptides. Illustrative of such libraries include cDNA and genomic DNA libraries, combinatorial and peptide expression libraries.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1E are drawings showing the nucleotide sequence of SYNAP-a coding (SEQ ID NO. 1) and non-coding (SEQ ID NO.2) strands. The predicted amino acid sequence is also shown (SEQ ID. NO. 3).

20 Figures 2A-2E are drawings showing the nucleotide sequence of SYNGAP-b coding (SEQ ID NO. 4) and non-coding (SEQ ID NO. 5) strands. The predicted amino acid sequence is also shown (SEQ ID. NO. 6).

Figures 3A-3E are drawings illustrating the nucleotide sequence of SYNGAP-c
25 coding (SEQ ID NO. 7) and non-coding (SEQ ID NO. 8) strands. Also shown is the amino acid sequence (SEQ ID NO. 9) and an untranslated amino acid sequence (SEQ ID NO. 8A) following the C-terminus of the predicted SYNGAP-c protein.

Figure 4A is a drawing showing the predicted amino acid sequence of SYNGAP-c
30 (SEQ ID NO. 9).

Figure 4B is a schematic drawing showing the domain organization of SYNGAP-c.

Figure 4C is a drawing showing a sequence alignment between the Ras-GAP domain of SYNGAP-c (SEQ ID NO. 10); *Rattus norvegicus* (rn) Ras-GAP (L13151; SEQ ID NO. 11) and *Homo sapiens* neurofibromin (hs 1NFI, M38107; SEQ ID NO. 12). Identical residues are in bold type. Hyphens designate conceptual amino acid sequence deletions to maximize alignment.

Figure 5 is a drawing showing a sequence alignment between the pleckstrin homology (PH) of SYNGAP-a (SEQ ID NO. 13); *Homo sapiens* p120 RasGAP (Hs p120 Genbank Accession No. P20936; SEQ ID NO. 14); *D. melanogaster* GAP1 (Dm GAP1 Genbank Accession No. P48423); and *Homo sapiens* pleckstrin (Hs Plec Genbank Accession No. P08567). Dots designate conceptual amino acid sequence deletions to maximize alignment.

Figure 6 is a drawing showing a sequence alignment between the C2 domain of SYNGAP-a (SEQ ID NO. 17); *Homo sapiens* p120 RasGAP (Hs p120 Genbank Accession No. P20936; SEQ ID NO. 18); *Rattus norvegicus* Synaptotagamin II (Genbank Accession No. P29101; SEQ ID NO. 19); and *Bos taurus* Rabphilin 3A (Genbank Accession No. A48097; SEQ ID NO. 20). Dots designate conceptual amino acid sequence deletions to maximize alignment.

Figure 7A is a representation of a Northern blot showing high level of SYNGAP mRNA expression in the brain.

Figure 7B is a representation of a Western blot showing SYNGAP in rat tissues using immunoblot techniques. These antibodies recognized a 130kDa protein in HEK293T cells transfected with the SYNGAP cDNA (Lane 2) and in rat brain

homogenates (Lane 3). The 130kDa protein was not detected in mock transfected HEK293T cells (lane 1) and immunorecognition of the 130kDa protein in brain was blocked by preabsorbtion of the antibody with the antigen (Lane 4).

5 Figure 7C is a representation of a Western blot showing SYNGAP in rat tissues using the SYNGAP antibody. Expression of the SYNGAP protein is brain specific.

10 Figure 7D is a representation of an immunoblot showing that rat brain cortex crude homogenates (cortex) and cytosolic and membrane fractions include membrane-associated SYNGAP. Cortical membranes were extracted with the indicated detergents and then separated into soluble (S) and pellet fractions (P) and the distribution of SYNGAP detected using immunoblot techniques. SYNGAP is resistant to membrane extraction with non-denaturing detergents similar to other proteins located in the PSD fraction.

15 Figure 8 is a representation of an immunoblot illustrating that SYNGAP is associated with the ONEIDA receptor complex. In this figure, immunoprecipitates were analyzed for the presence of SYNGAP, NMDAR1, PSD-95 and GluR1.

20 Figures 9A-9F are representations of photomicrographs showing that SYNGAP is localized to synapses.

25 Figures 10A-10I are representations of photomicrographs showing that SYNGAP is specifically localized to excitatory synapses.

 Figure 11 is a graph showing that the GAP domain of SYNGAP stimulates H-Ras GTPase activity.

DETAILED DESCRIPTION OF THE INVENTION

As summarized above, the present invention features molecules that relate to mammalian SYNGAP and methods of using such molecules. Particularly provided are novel polynucleotides and polypeptides that can be used in the methods. Further provided are methods for isolating the molecules and using same to treat or prevent disorders relating to SYNGAP. Additionally provided are screening methods that can be employed to detect compounds with capacity to diagnose or treat disorders associated with SYNGAP.

In general, optimal practice of the present invention can be achieved by use of recognized manipulations such as those involving molecular, cell culture, biochemical and electrophysiological techniques. For example, techniques for and purifying nucleic acids, methods for making and screening cDNA libraries, methods for making recombinant vectors, cleaving DNA with restriction enzymes, ligating DNA, introducing DNA into host cells by stable or transient means, culturing the host cells, methods for isolating and purifying polypeptides, methods for making antibodies, methods for assaying signal transduction molecules, methods for detecting protein binding and particularly binding to PDZ and receptor domains, computer-assisted methods for detecting nucleic acid or amino acid sequence homologies, and electrophysiological techniques are generally known in the field. See generally Sambrook et al., *Molecular Cloning* (2d ed. 1989); Ausubel et al., *Current Protocols in Molecular Biology*, (1989) John Wiley & Sons, New York; S. Altschul et al. *Nuc. Acids Res.*, 25: 3389-3402 (1997); Marshall, C.J. *Current Opin. in Cell Biol.* (1996) 8: 197; Sheng, M. *Neuron* (1996) 17: 575 and references cited therein.

Unless otherwise indicated, reference to SYNGAP means a polynucleotide or amino acid sequence that is substantially homologous to at least one of SYNGAP-a, SYNGAP-b, or SYNGAP-c. Preferred use of the term SYNGAP means SYNGAP-a, SYNGAP-b or SYNGAP-c. As noted, SYNGAP-a, SYNGAP-b, and SYNGAP-c are alternatively spliced variants. Sometimes SYNGAP-c will be specifically referred to herein as "GAPSYN".

Polynucleotides of this invention can be derived from a variety of sources such as a mammalian source and particularly a rodent or a primate source, e.g., rat, rabbit, mouse or human source. It will be appreciated that the present disclosure provides ample information to facilitate isolation of a variety of mammalian SYNGAPs through use of recognized molecular techniques such as the Polymerase Chain Reaction (PCR) amplification and related amplification techniques. Typically, the isolated polynucleotide will be positioned in a recombinant vector, although in some cases it may be desirable to provide the polynucleotide without a recombinant vector, e.g., as a PCR-amplified product. Particularly, the polynucleotide can be provided in a suitable DNA vector capable for expressing an encoded SYNGAP or a fragment or derivative thereof in a eukaryotic or prokaryotic cell expression system. The polynucleotide may include operably linked transcriptional elements such as a promoter, leader and optimal enhancer sequences to drive expression of the encoded polypeptide in a desired host cell expression system. Alternatively, the DNA vector itself may provide some or all of the control elements. In general, polynucleotides of the invention that encode SYNGAP including fragments and derivatives thereof, are often made so that naturally-occurring SYNGAP control sequences (e.g., genomic control sequences) are reduced in number and preferably removed.

As will be apparent from the preceding discussion, the ability to detect and modulate SYNGAP *in vitro* and *in vivo* is very important. For example, inappropriate SYNGAP activity may negatively impact synaptic function, signal transduction or both. That inappropriate SYNGAP activity may arise from one or a combination of different causes such as a genetic deficiency, chronic illness, viral infection, bacterial infection, trauma (e.g., emotional or physical) and the like. In particular, relationship between synapse function, receptor function and signal transduction may be adversely affected by the inappropriate SYNGAP activity. Thus, the present methods are particularly useful for diagnosing and treating disorders arising from unsuitable SYNGAP activity.

By the term "SYNGAP activity" or like term is meant those functions attributed to SYNGAP as discussed herein, e.g, PDZ domain and rasGTPase inhibition. It will be appreciated that related activities can impact SYNGAP activity including synthesis of SYNGAP (transcription and translation), SYNGAP processing (e.g., protein maturation including modification such as glycosylation), protein stability in SYNGAP-expressing cells, and neuromodulation.

As discussed above, the present invention provides methods to detect mammalian SYNGAP *in vitro* or *in vivo*. Further provided are useful methods for modulating, including enhancing, expression or activity of SYNGAP in particular cells such as those that include chemical synapses with SYNGAP. By way of illustration, one can provide an anti-sense SYNGAP molecule to neurons to selectively inhibit SYNGAP activity in those neurons. In addition, a suitable SYNGAP antibody or antigen-binding fragment thereof can be provided to reduce or eliminate SYNGAP function. Further, compounds identified by the methods of this invention can be administered *in vitro* or *in vivo* e.g., to enhance SYNGAP function including increasing the number or quality of chemical synapses that include SYNGAP.

In general, therapeutic methods of this invention include administration of a therapeutically effective amount of a SYNGAP or SYNGAP-related molecule to a subject and particularly a human patient in need of such treatment. Therapeutic methods of the invention also include administration of an effective amount of compound identified by this invention to the subject, in need of such treatment for an indication as disclosed herein.

Illustrative subjects for the purposes of this invention include those mammals suffering from or susceptible to those conditions generally discussed above, ie. disorders of the CNS and PNS such as an affective disorder, cognitive disorder, or a neurodegenerative disorder. In particular, a wide variety of CNS disorders may be alleviated by selectively enhancing or inhibiting SYNGAP activity in the CNS and

particularly in the brain. As will be shown below, SYNGAP is predominantly expressed in the brain. Illustrative CNS disorders are affective disorders (e.g., depression), disorders of thought (e.g., schizophrenia) and degenerative disorders, as well as disorders manifested by application of anesthesia. CNS disorders of severe impact include pre-
5 senile dementia (sometimes referred to as Alzheimer's disease (AD) or early-onset Alzheimer's disease), senile dementia (dementia of the Alzheimer's type), Parkinson's disease (PD), and Huntington's disease (HD, sometimes referenced as Huntington's chorea). Such CNS disorders are well-represented in the human population. See generally; Gusella, J.F. et al. (1983) *Nature* 306: 234; Borlauer. W. and Jprmuloewoca. P.
10 (eds.) (1976); *Adv. in Parkinsonism: Biochemistry, Physiology, Treatment. Fifth International Symposium on Parkinson's Disease* (Vienna) Basel: Roche; and references cited therein. Subjects that have suffered acute CNS trauma also may be treated in accordance with the invention, e.g. brain or spinal cord ischemia or trauma, stroke, heart attack or neorological deficits that may be associated with surgery.

15

In the methods of the invention, a desired therapuetic molecule (ie. a suitable SYNGAP or SYNGAP-related molecule or identified compound) can be administered to a subject in need of treatment or suspected of needing treatment in any of several ways. For example, a desired SYNGAP or SYNGAP-related polynucleotide, immune system
20 molecule or a therapuetic compound can be administered as a prophylactic to prevent the onset of or reduce the severity of a targeted condition. Alternatively, the therapuetic molecule can be administered during or following the course of a targeted condition.

More specifically, the therapuetic molecule can be administered to a subject,
25 either alone or in combination with one or more therapeutic agents, as a pharmaceutical composition in mixture with conventional excipient, i.e. pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral or intranasal application which do not deleteriously react with the active compounds and are not deleterious to the recipient thereof. Suitable pharmaceutically acceptable carriers include
30 but are not limited to water, salt solutions, alcohol, vegetable oils, polyethylene glycols,

gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethyl-cellulose, polyvinylpyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously react with the active compounds.

Such compositions may be prepared for use in parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; intranasally, particularly in the form of powders, nasal drops, or aerosols; vaginally; topically e.g. in the form of a cream; rectally e.g. as a suppository; etc.

The pharmaceutical agents may be conveniently administered in unit dosage form and may be prepared by any of the methods well known in the pharmaceutical arts, e.g., as described in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., Easton, PA, 1980). Formulations for parenteral administration may contain as common excipients such as sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. In particular, biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be useful excipients to control the release of certain of the compounds.

Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration

may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration. Other delivery systems will administer the therapeutic agent(s) directly, e.g., by use of stents.

5 A therapeutic molecule of this invention can be employed in the present treatment methods as the sole active pharmaceutical agent or can be used in combination with other active ingredients, e.g., those compounds known in the field to be useful in the treatment of cognitive and neurological disorders.

10 The concentration of one or more treatment compounds in a therapeutic composition will vary depending upon a number of factors, including the dosage of the therapeutic compound to be administered, the chemical characteristics (e.g., hydrophobicity) of the composition employed, and the intended mode and route of administration. In general terms, one or more than one of the therapeutic compounds
15 compounds may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v of a compound for parenteral administration. As noted above, GAPYSN antibodies and antigen-binding fragments thereof can be modified according to standard methods to deliver useful molecules or can be modified to include detectable labels and tags to facilitate visualization of synapses including SYNGAP.

20 It will be appreciated that the actual preferred amounts of active compounds used in a given therapy will vary according to e.g. the specific compound being utilized, the particular composition formulated, the mode of administration and characteristics of the subject, e.g. the species, sex, weight, general health and age of the subject. Optimal
25 administration rates for a given protocol of administration can be readily ascertained by those skilled in the art using conventional dosage determination tests conducted with regard to the foregoing guidelines. Suitable dose ranges may include from about 1µg/kg to about 100mg/kg of body weight per day.

Therapeutic compounds identified by the present methods can be suitably administered by conventional routes. For example, when the therapeutic compound is a synthetic or naturally-occurring chemical compound such as a drug, it will be preferred to administer the compound in a protonated and water-soluble form, e.g., as a

5 pharmaceutically acceptable salt, typically an acid addition salt such as an inorganic acid addition salt, e.g., a hydrochloride, sulfate, or phosphate salt, or as an organic acid addition salt such as an acetate, maleate, fumarate, tartrate, or citrate salt.

Pharmaceutically acceptable salts of therapeutic compounds of the invention also can include metal salts, particularly alkali metal salts such as a sodium salt or potassium salt; 10 alkaline earth metal salts such as a magnesium or calcium salt; ammonium salts such as an ammonium or tetramethyl ammonium salt; or an amino acid addition salts such as a lysine, glycine, or phenylalanine salt.

Current therapeutic practice typically utilizes one or a combination of different 15 drugs to treat the disorders described above. As noted, the present invention provides methods for detecting compounds capable of treating or preventing the disorders. Compounds identified by these methods may be used either alone, or in combination with currently used therapies to alleviate the disorders or to reduce symptoms associated with the disorders. In particular, specific drugs that have been reported to be of use in the 20 treatment of affective disorders, e.g., depression, manic-depressive disorders, anxiety disorders such as panic attacks and the like. Many of these drugs have been reported to work by modulating synaptic function, e.g., by altering receptor activity. According to methods of the present invention, compounds with capacity to modulate neuroreceptors, e.g., by increasing SYNGAP activity, may be similarly effective at treating depressive 25 disorders. Such compounds may be identified by practice of the screening methods described herein.

Compounds identified by the methods of the invention can be further tested if desired in standard assays used to measure higher nervous system functions such as 30 habituation, sensitization, learning and memory. Examples of such systems include those

using well known test organisms such as *Aplysia*, *C. elegans*, *D melanogaster*, primates such as monkeys, and rodents such as mice, rabbits and rats. Preferred compounds are those that can increase or decrease at least one of these functions by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% up to about 100% as determined by a
5 suitable testing protocol recognized in the test organism selected.

Compounds identified by the present methods can be administered to a subject and preferably a human patient suffering from or suspected of suffering from a SYNGAP-related disorder as described above.

10

Particularly preferred systems for performing the testing methods of this invention involve cell culture assays and especially cell culture assays employing primary or cultured cells derived from the nervous system. Preferred cultured cells are capable of expressing or express excitatory chemical synapses including SYNGAP such as CNS-
15 derived cells such as those derived from the brain. Illustrative cells include are provided below in the examples. If desired, a cultured cell line can be tested for SYNGAP expression by determining if the cells express or can be made to express SYNGAP. Methods for detecting expression include immunological methods involving a suitable SYNGAP antibody, e.g., a Western blot, RIA, ELISA or other immunoassay.

20

In addition to the specific CNS- and PNS-related applications described herein, the present invention can also be used to therapeutically intervene in other systems that are affected by inappropriate SYNGAP activity. Such systems include the endocrine system for treatment of hormonal imbalances, the immune system for intervention in
25 antigen processing, secreted immunomodulators, and viral processing, as well as anti-tumor applications, such as regulation of synapse formation in malignancies of the neuroendocrine system. To reduce or avoid CNS-or PNS-related side-effects, compounds identified in the methods of this invention may be re-screened multiple times, e.g., 2, 3, 4, or 5 times to identify compounds that specifically modulate SYNGAP in the neurons.

30

As discussed, the present invention provides isolated polynucleotides that encode SYNGAP or a fragment or a derivative of SYNGAP. The isolated polynucleotides may be cloned or subcloned using nearly any method known in the art. See e.g., Sambrook, J. et al., *supra*. In particular, nucleotide sequences of the invention may be cloned into any
5 of a large variety of vectors. Possible vectors include, but are not limited to, cosmids, plasmids or modified viruses, although the vector system must be compatible with the host cell used. Viral vectors include, but are not limited to, lambda, simian virus, bovine papillomavirus, Epstein-Barr virus, and vaccinia virus. Viral vectors also include retroviral vectors, such as Amphotrophic Murine Retrovirus (see Miller et al.,
10 *Biotechniques*, 7:980-990 (1984)), incorporated herein by reference). Plasmids include, but are not limited to, pBR, pCMV5, PUC, pGEM (Promega), and Bluescript™ (Stratagene) plasmid derivatives. Introduction into and expression in host cells is done for example by, transformation, transfection, infection, electroporation, etc. See the examples which follow for particularly preferred recombinant vectors.

15 For preferred production of anti-sense RNA, use of specified recombinant vectors typically including strong bacterial or eukaryotic (e.g., viral) promoters will usually be desired. See e.g., Ausubel et al. *supra*.

20 The term "vector" or "recombinant vector" as used herein means any nucleic acid sequence of interest capable of being incorporated into a host cell and resulting in the expression of a nucleic acid sequence of interest. Vectors can include, e.g., linear nucleic acid sequences, plasmids, cosmids, phagemids, and extrachromosomal DNA. Specifically, the vector can be a recombinant DNA. Also used herein, the term
25 "expression" or "gene expression", is meant to refer to the production of the protein product of the nucleic acid sequence of interest, including transcription of the DNA and translation of the RNA transcript. Most recombinant vectors will include a "cloning site" which as used herein is intended to encompass at least one restriction endonuclease site. Typically, multiple different restriction endonuclease sites (e.g., a polylinker) are
30 contained within the vector to facilitate cloning.

As noted, preferred polynucleotides of this invention encode a mammalian SYNGAP having a molecular of between about 100, 110, 120, 130, 140, or about 150 kDA or greater. Also preferred are those polynucleotides that are at least 70%, 75%, 80%, 90%, 95%, 99% or greater sequence identity to any of the nucleotide sequences specifically shown in SEQ ID NOS: 1-2, 4-5, or 7-8. As will be fully appreciated, such sequences are substantially homologous to the sequences shown in SEQ ID NOS: 1-2, 4-5, or 7-8. A more preferred polynucleotide of the invention encodes the rat SYNGAP shown in SEQ ID NOS. 3, 6 or 9. A specifically preferred polynucleotide encodes the rat SYNGAP shown in SEQ ID NO. 9. See Figures 1A-1E; 2A-2E; and 3A-3E.

By the term "substantially homologous" is meant relationship between two nucleic acid molecules and generally refers to subunit sequence similarity between the two molecules. Typically, the two nucleic acid molecules will be DNA. When a subunit position in both of the DNA molecules is occupied by the same monomeric subunit, i.e. a nucleotide, then they are homologous at that position. Homology between the two sequences is a direct function of the number of matching or homologous positions, e.g., if 50% of the subunit positions in the two DNA sequences are homologous then the two sequences are 50% homologous. By "substantially homologous" is meant largely but not wholly homologous. More particularly, the term is meant to denote at least about 70% or greater homology as defined above with respect to the rat SYNGAP sequences illustrated in SEQ ID NOS: 1-2, 4-5, or 7-8. Preferred nucleotide sequences of the invention have at least about 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% homology as defined above with respect to the rat SYNGAP sequences illustrated in SEQ ID NOS: 1-2, 4-5, or 7-8.

The term "substantially homologous" is also used herein with reference to relationship between two polypeptide sequences and generally refers to subunit sequence similarity between the two molecules. When a subunit position in both of the polypeptides is occupied by the same monomeric subunit, i.e. an amino acid sometimes referred to as an amino acid residue, then they are homologous at that position. Homology

between the two sequences is a direct function of the number of matching or homologous positions, eg., if 50% of the subunit positions in the two polypeptides are homologous then the two sequences are 50% homologous. By "substantially homologous" is meant largely but not wholly homologous. More particularly, the term is meant to denote at least
5 about 70% or greater homology as defined above with respect to the rat SYNGAP sequences illustrated in SEQ ID NO: 3, 6, or 9. Preferred polypeptides of the invention have at least about 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% homology as defined above with respect to the rat SYNGAP sequences illustrated in SEQ ID NO: 3, 6, or 9.

10 Two substantially homologous polynucleotides can be identified by one or a combination of different strategies. For example, in one approach, a polynucleotide of this invention that is substantially homologous to any one of sequences shown in SEQ ID NOs: 1-2, 4-5, or 7-8 in addition to fragments and derivatives thereof of a length
15 sufficient to bind to the sequences in the sequences, can be identified by employing moderately stringent conditions. In particular, moderate stringency conditions are meant to include a hybridization buffer comprising about 20% formamide in 0.8M saline/0.08M sodium citrate (SSC) buffer at a temperature of 37°C and remaining bound when subject to washing once with that SSC buffer at 37°C. Additionally, highly stringent conditions are meant to include a hybridization buffer comprising about 50% formamide in SSC
20 buffer at about 42 °C and remaining bound when washed in SSC buffer. See e.g., Sambrook et al. *supra*.

Additional methods of detecting and quantitating substantial homology refer to so-called "dry" methods and include use of publically available computer programs that
25 can readily determine homology between nucleic acids of known or partially known sequence. Exemplary of such programs include the BLAST program available from the National Library of Medicine (Genbank). See also S. Altschul et al. *J. Mol. Biol.*, 215:403-410 (1990); and S. Altschul et al. *Nuc. Acids Res.*, 25: 3389-3402 (1997) for specific disclosure relating to use of the BLAST program.

Nucleic acid fragments and derivatives of this invention preferably should comprise at least about 12 to about 50 nucleotides, at least about 60, 100 to 200 nucleotides, at least about 300, 400, to about 500 nucleotides, or at least about 1000, 1500, 2000, 2500, 3000, 3500 to about 4000 nucleotides or more. In some preferred
5 embodiments, the nucleic acid fragment or derivative is bound to a suitable moiety, sometimes called a tag, which permits ready identification such as a radionucleotide, fluorescent or other chemical identifier.

The polynucleotide sequences of the invention can be altered by mutations such as
10 substitutions, additions or deletions (contiguous or non-contiguous) that can provide for substantially homologous nucleic acid sequences. In particular, a given nucleotide sequence can be mutated *in vitro* or *in vivo*, to create variations in the nucleotides, e.g., to form new or additional restriction endonuclease sites or to destroy preexisting ones and thereby to facilitate further *in vitro* modification. Any technique for mutagenesis known
15 in the art can be used including, but not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., *J. Biol. Chem.*, 253:6551 (1978)), use of TAB Registered TM linkers (Pharmacia), PCR-directed mutagenesis, and the like.

It will be appreciated that due to the degeneracy of genetic code , a number of
20 different nucleic acid sequences may be used in the practice of the present invention. This includes the substitution of different codons encoding the same amino acid residue within the sequence, thus producing a silent or nearly silent change. Almost every amino acid except tryptophan and methionine is represented by several codons. Often the base in the third position of a codon is not significant, because those amino acids having 4 different
25 codons differ only in the third base. This feature, together with a tendency for similar amino acids to be represented by related codons, increases the probability that a single, random base change will result in no amino acid substitution or in one involving an amino acid of similar character. See generally Alberts et al., *Molecular Biology of the Cell*, (1989) Garland Publishing, New York.

Thus, the present invention includes polynucleotides with genetic alterations that do not substantially impact SYNGAP function as related herein. The genetic alterations can be synthetic, i.e., can be introduced experimentally, or may be naturally-occurring, e.g., in the form of SYNGAP isoforms or allelic variants. Additionally, SYNGAP-a, SYNGAP-b, and SYNGAP-c are illustrative of naturally-occurring splice variants. See Figures 1A-1E; 2A-2E; and 3A-3E.

As noted, the present invention provides oligonucleotide primers that are complementary to any of the nucleotide sequences shown in SEQ ID NOs: 1-2, 4-5, or 7-8. In most cases, the primers will be a DNA sequence of between about 12 to about 70 nucleotides in length preferably about 20, 30, 40, to about 50 or about 55 nucleotides in length. The oligonucleotide primers can suitably include restriction sites to add specific restriction enzyme cleavage sites to the PCR product as needed, e.g., to introduce a ligation site. Preferred DNA oligonucleotide primers are spaced from one another in opposing direction relative to extension of the primers. That is, the primers are spaced relative to each other on a polynucleotide template (usually on different strands) sufficient to produce an amplification product of at least about 50 nucleotides, at least about 60 to about 100 nucleotides, at least about 200 to 500 nucleotides, at least about 600 to 1000 nucleotides, or at least about 1000 to 3000 nucleotides up to about 4000 nucleotides as determined, e.g., by gel electrophoresis. Synthetic methods for making oligonucleotide primers are well known in the field. Exemplary primers are provided in the examples which follow.

Preferred polynucleotides of this invention are capable of modulating and preferably inhibiting Ras by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, up to about 100% in what has been referred to as a standard Ras GTPase assay. A variety of Ras GTPase assays are known in the field. For example, a preferred standard assay includes at least one of the following general steps:

- a) mixing Ras or a GTPase- active portion of Ras with a suitable amount of detectably-labeled GTP (e.g., $\gamma^{32}\text{P}$ -GTP],

- c) contacting the mixture with a mammalian SYNGAP, e.g., the amino acid sequence shown in SEQ ID NO: 3 or a fragment thereof including at least a Ras-GTPase active portion of the SYNGAP (ie., the GAP domain); and
- d) detecting binding of the detectably-labeled GTP to the Ras or Ras portion.

5

As will be shown below, the Ras and/or the SYNGAP protein can be fused (ie. covalently linked) to a suitable protein tag such as GST to facilitate protein isolation and purification. If desired, the above general method can be adapted to include a suitable control which can be a GST-fusion protein such as those known in the field. In general, the control is prepared and incubated under the same or closely related conditions as the assay but does not include addition of the SYNGAP or fragment thereof. A particularly preferred method for detecting Ras-GTPase activity involves measuring $\gamma^{32}\text{P}$ -GTP over a time period of between from about 30 seconds to about one hour or longer, typically from about 1 minute to about 20 minutes or longer. See Settleman et al. (1992) *Cell*. 69:539.

15

By the term "Ras-GTPase" portion of SYNGAP is meant at least the SYNGAP Ras-GTPase domain which domain is capable of negatively regulating Ras as determined by the standard Ras-GTPase assay described herein. Additional SYNGAP Ras-GTPase domains are those domains that are substantially homologous to the GAP domain shown between amino acid residues 266 and 502 of the rat SYNGAP sequence. See Figure 4C and SEQ ID No. 11. Preferred Ras-GTPase portions have a length of between about 200, 300 to about 400 amino acids and include amino acid residues (bold) shown in 4C (GAPSYN). The bolded amino acids shown in Figure 4C will typically be present in the Ras-GTPase portion in the alignment shown.

25

By the term "pleckstrin homology" (PH) is meant an amino acid sequence that is substantially homologous to any one of the following amino acid sequences: SynGAP-a, 162 to 243; SYNGAP-b, 60-141; and SYNGAP-c, 4 to 85. See Figures 1A-1E; 2A-2E; and 3A-3E; and 4A-B. Preferred PH domains have a length of between about 20, 30, 40, 50, 60, 70, 80, 90, to about 100 amino acids and include amino acid residues (bold)

30

shown in Figure 5 (SYNGAP-a). The bolded amino acids shown in Figure 5 will typically be present in the PH domain in the alignment shown.

By the term "C2 domain" is meant an amino acid sequence that is capable of
5 modulating phospholipid-dependent calcium (Ca^{+2}) binding to the SYNGAP as
determined by assays disclosed herein. Preferred are those sequences which are
substantially homologous to any one of the following amino acid sequences: SynGAP-a,
245 to 354; SYNGAP-b, 143 to 252; and SYNGAP-c, 87 to 196. See Figures 1A-1E;
2A-2E; 3A-3E and 4A-B. Additionally preferred C2 domains have a length of between
10 about 90, 100, 120, 130, up to about 150 and include amino acid residues (bold) shown in
Figure 6 (SYNGAP-a). The bolded amino acids shown in Figure 6 will typically be
present in the PH domain in the alignment shown.

A variety of phospholipase C assays are known in the field which assays can be
15 readily adapted in accord with the present invention. See e.g., James, S.R. et al., (1997)
Cell Signal. 329; and Hurley, J.H. (1997) *Curr. Opin. Struct. Biol.* 7:557; and references
cited therein.

The RasGTPase activating activity of SYNGAP can be measured by a variety of
20 means including use of the MAP kinase cascade as a reporter in a primary or
immortalized cell culture, e.g., hippocampal neurons or HEK 293, NIH3T3 cells. The
regulation of Ras activity by SYNGAP in a selected cell or cell line can be observed by
transfecting the cells with a SYNGAP cDNA plasmid with, e.g., a luciferase or other
suitable reporter construct that is activated by the MAP kinase cascade. See e.g., 1997
25 instruction manual entitled *PathDetectTM In Vivo Signal Transduction Pathway Trans-
Reporting Systems*, pp.1-20 (Stratagene Cloning Systems, La Jolla, Ca). Alternatively,
SYNGAP cDNA can be transfected into primary culture cells with the reporter construct.
SYNGAP activity can then be indirectly assayed by quantifying luciferase (or other
reporter activity) with a luminometer or other suitable measuring device. Various agents,
30 e.g., drugs, that regulate SYNGAP activity of the pathway can be rapidly screened by this

method. For example, a variety of drugs such as growth factors, cytokines, neurotransmitters and the like can be tested for capacity to regulate SYNGAP. Importantly, agents that activate or inhibit SYNGAP activity can be rapidly analyzed.

5 Additionally preferred polynucleotides of this invention encode a mammalian SYNGAP or SYNGAP-related amino acid sequence that is capable of binding 1, 2, 3, up to about 10 PDZ domains as determined by a standard PDZ domain binding assay. For example, a preferred standard PDZ domain binding assay involves what has been referred to as the yeast two-hybrid system.

10 In general, the yeast GAL4 two hybrid system can detect protein-protein interactions based on the reconstitution of function of GAL4 (yeast transcriptional activator) by activation of a GAL1-lacZ reporter gene. Like several many other transcription activating factors, the GAL4 protein contains two distinct domains, a DNA
15 binding domain and a transcription activation domain. Each domain can be independently expressed as a portion of a fusion protein composed of the domain, and a second, "bait" interacting protein. The two fusion proteins are then independently expressed together in a cell. When the two GAL4 domains are brought together by a binding interaction between the two "interacting" proteins, transcription of a reporter gene under the
20 transcriptional control of GAL4 is initiated. The reporter gene typically has a promoter containing GAL4 protein binding sites (GAL upstream activating sequences, UAS[G]). Several examples of yeast two-hybrid assays have been reported. See e.g., U.S. Pat. Nos. 5,693,476 and 5,695,941.

25 A two hybrid system such as is described above may be used to identify proteins and particularly synaptic proteins that include at least one suitable SYNGAP-binding PDZ domain. As noted above and more specifically in the following discussion, that interaction is believed to occur due to binding between the PDZ domain and the C-terminal sequence ((Tor S), X V) at the end of SYNGAP wherein X is any of the 20
30 common amino acids (Ala, Arg, Asn, Asp, Lys, Gln, Glu, Gly, His, Ile, Lev, lys, Met,

Phe, Pro, Ser, Thr, Trp, Tyr, Val). A specific PDZ binding assay involves at least one of the following steps:

a) fusing a polynucleotide encoding SYNGAP or a suitable fragment thereof to the GAL4 DNA binding domain (G4BD) (fusion protein) in a suitable yeast expression vector,

b) transforming the vector carrying fusion protein into yeast cells harboring a suitable reporter gene activated by GAL4 (e.g., a suitable LacZ construct),

c) transforming the cells with a second vector carrying a fusion between a polypeptide comprising at least one PDZ domain (e.g, PSD-95/SAP90) and the transcription activating domain of yeast GAL4; and

d) screening the transformants for expression of the reporter gene in the yeast.

In most cases, the yeast transformants are screened using a beta -galactosidase (beta -gal) assay on plates containing the chromogenic substrate X-gal. Reporter-expressing cells can be selected, cloned, and analyzed if desired. It may be of interest in some instances to use a second two hybrid system, described in detail in Ausubel, et al., that utilizes a native E. coli LexA repressor protein. That protein binds tightly to appropriate operators. A plasmid is used to express one of a pair of interacting proteins (the "bait" protein) as a fusion to LexA. Example 2 below describes an especially preferred assay for detecting PDZ binding to SYNGAP using a yeast two-hybrid system.

Preferred polynucleotides are capable of encoding sequence that result in at least about a 10% to 20% up to about a 100% increase or more of positive (blue) colonies relative to a suitable control. Colony number can be determined by inspection.

Additional standard assays can be conducted to detect PDZ binding to SYNGAP and SYNGAP- related polypeptide s of this invention. For example, specific biochemical assays are useful for detecting PDZ interactions including certain partner assays. For example, a polynucleotide can be expressed and the encoded polypeptide immobilized on a suitable solid support. A suitable PDZ protein such as PSD-95/SAP90 or a fragment or

a derivative thereof is contacted to the solid support under conditions conducive to binding between the PDZ protein and the SYNGAP or SYNGAP-related polypeptide . Typically, the PDZ protein will be detectably-labeled, e.g., with a radionuclide or other suitably detectable tag to facilitate detection and quantitation, if desired, of the binding.

5 Preferred methods for detecting the binding include well known panning methods, e.g., immunopanning, preferably conducted in multi-well plates. Alternatively, the binding can be assayed by conventional immunological techniques such as RIA, ELISA, affinity chromatography, Western blots, etc. Preferred polynucleotides are capable of encoding polypeptides that exhibit and increase in binding to the PDZ protein of at least about
10 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, up to 100% or more relative to a suitable control polypeptide that does not include a detectable PDZ domain (e.g, LacZ).

Additionally preferred polynucleotides of this invention are capable of encoding polypeptides that bind PDZ domains as detected by use of affinity biosensor methods.

15 Such methods may be based on a variety of detectable effects including surface plasmon resonance (SPR). SPR is particular advantageous for monitoring molecular interactions in real-time, enabling a sensitive and comprehensive analysis of the effects of test compounds on the binding interactions between two proteins than the methods discussed above.. Preferred polynucleotides of this invention are capable of encoding polypeptides
20 that are capable of binding proteins comprising at least one PDZ domain and causing a change in reflectance intensity of at least about 10% to about 20% relative to a suitable control protein (e.g., LacZ). See e.g., Cullen, D. C., et al., *Biosensors* 3:211-225 (1988).

The above-mentioned methods can be readily adapted to detect compounds that
25 can modulate SYNGAP activity, e.g., by modulating SYNGAP binding to a polypeptide comprising one or more PDZ domains, modulating SYNGAP-mediated Ras GTPase activity, or both. In general, any of the methods can be optimized to include addition of one or more test compounds optionally in parallel with a suitable control, to detect modulation.

A variety of different compounds may be screened using methods of the present invention. They include peptides, macromolecules, small molecules, chemical and/or biological mixtures, and fungal, bacterial, or plant extracts. Such compounds, or molecules, may be either biological, synthetic organic, or even inorganic compounds, and
5 may be obtained from a number of sources, including commercial or publically-accessible vendors of libraries of compounds.

In cases where an identified compound is a peptide, the peptide may be utilized to aid in the discovery of small molecule mimetics. Methods of the present invention are
10 well suited for screening libraries of compounds in multi-well plates (e.g., 96-well plates), with a different test compound in each well. In particular, the methods may be employed with combinatorial libraries. A variety of combinatorial libraries of random-sequence oligonucleotides, polypeptides, or synthetic oligomers have been proposed including small-molecule libraries. Alternatively, the library may be formed by solid-
15 phase synthetic methods in which beads containing different-sequence oligomers that form the library are alternately mixed and separated, with one of a selected number of subunits being added to each group of separated beads at each step. The identity of library compounds with desired effects on the binding of an SYNGAP and a protein comprising one or more PDZ domains such as PSD-95/SAP90 can be determined by any
20 of the methods described herein. See e.g., Houghten, R. A., (1985) *PNAS (USA)* 85:5131.

As noted, particularly preferred polynucleotides of this invention exhibit a length of between about 50 to about 100 nucleotides, about 200, 300, 400, 500, 600, 800, 1000,
25 1500, 2000, 2500, 3000, 3500, 4000 or more as determined by standard nucleic acid sizing techniques such as agarose or polyacrylamide gel electrophoresis. The polynucleotide can be RNA, DNA or a chimera thereof as desired.

Exemplary host cells which can express the isolated polynucleotides of this
30 invention are known in the field and include bacterial cells (e.g., *E. coli*) such as MM294,

DM52, XL1-blue (Stratagene) strains of *E. coli*, and animal cells (e.g., CV-1 and COS-7 cells). In addition, it is possible to express certain isolated nucleic acids of the invention in certain yeast cells (e.g., *S. cerevisiae*), amphibian cells (e.g., *Xenopus oocyte*), and insect cells (e.g., *Spodoptera frugiperda* and *Trichoplusia ni*). Methods for expressing
5 isolated and recombinant DNA in these cells are known. See e.g., Sambrook et al., *Molecular Cloning* (2d ed. 1989), Ausubel et al. *supra*, and Summer and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*: Texas Agricultural Experimental Station Bulletin No. 1555, College Station Texas (1988). Specifically preferred host cells are discussed more fully below.

10

The polynucleotides of this invention can be readily made by techniques well known in the field including those techniques involving large-scale production thereof such as those including use of roller bottles, bioreactors and the like.

15

The term "complementary" or like term refers to the hybridization or base pairing between nucleotides or nucleic acids, such as, for instance, between the two strands of a double stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single stranded nucleic acid to be sequenced or amplified.

Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two single
20 stranded RNA or DNA molecules are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 95% of the nucleotides of the other strand, usually at least about 98%, and more preferably from about 99 to about 100%. Complementary polynucleotide sequences can be identified by a variety of
25 approaches including use of well-known computer algorithms and software.

Polynucleotides of this invention are typically isolated, meaning that the polynucleotides usually constitute at least about 0.5%, preferably at least about 2%, and more preferably at least about 5% by weight of total polynucleotide present in a given
30 fraction. A partially pure polynucleotide constitutes at least about 10%, preferably at

least about 30%, and more preferably at least about 60% by weight of total nucleic acid present in a given fraction. A pure polynucleotide constitutes at least about 80%, preferably at least about 90%, and more preferably at least about 95% by weight of total polynucleotide present in a given fraction. Purity can be determined by standard methods including gel electrophoresis.

It is preferred that the polypeptides of the present invention be substantially pure. That is, the polypeptides have been isolated from cell constituents that naturally accompany it so that the polypeptides are present preferably in at least 80% or 90% to 95% homogeneity (w/w). Polypeptides having at least 98 to 99% homogeneity (w/w) are most preferred for many pharmaceutical, clinical and research applications. Once substantially purified the polypeptide should be substantially free of contaminants for therapeutic applications. Once purified partially or to substantial purity, the polypeptides can be used therapeutically, or in performing a desired assay. Substantial purity can be determined by a variety of standard techniques such as chromatography and gel electrophoresis.

Particularly preferred polynucleotides and polypeptides of this invention are provided as substantially sterile formulations.

As noted, illustrative polynucleotides of this invention include those encoding a mammalian SYNGAP as identified herein. Preferably the polynucleotides are at least substantially homologous to any of the sequences shown in SEQ ID NOs: 3, 6 or 9. For example, the mammalian SYNGAP can be derived from a primate such as a monkey or a human; or the mammalian SYNGAP can be derived from a rodent such as a rat, mouse, guinea pig, or rabbit. By the term "derived from" is meant that at least a portion of the polynucleotide was isolated from or copied from a naturally-occurring mammalian nucleic acid such as a genomic DNA or cDNA.

The SYNGAP and SYNGAP-related polypeptides of the present invention can be separated and purified by appropriate combination of known techniques. These methods include, for example, methods utilizing solubility such as salt precipitation and solvent precipitation, methods utilizing the difference in molecular weight such as dialysis, ultra-
5 filtration, gel-filtration, and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in electrical charge such as ion-exchange column chromatography, methods utilizing specific affinity such as affinity chromatograph, methods utilizing a difference in hydrophobicity such as reverse-phase high performance liquid chromatograph and methods utilizing a difference in isoelectric point, such as isoelectric focusing
10 electrophoresis, metal affinity columns such as Ni-NTA. See generally Sambrook et al. and Ausubel et al. *supra* for disclosure relating to these methods.

It is preferred that the polypeptides of this invention be substantially pure. That is, the fusion proteins have been isolated from cell constituents that naturally accompany
15 it so that the fusion proteins are present preferably in at least 80% or 90% to 95% homogeneity (w/w). Fusion proteins having at least 98 to 99% homogeneity (w/w) are most preferred for many pharmaceutical, clinical and research applications. Once substantially purified the fusion protein should be substantially free of contaminants for therapeutic applications. Once purified partially or to substantial purity, the soluble fusion
20 proteins can be used therapeutically, or in performing *in vitro* or *in vivo* assays as disclosed herein. Substantial purity can be determined by a variety of standard techniques such as chromatography and gel electrophoresis.

Preferred polypeptides of this invention generally exhibit a molecular weight from
25 about 100, 110, 120, 130, 140, to about 150 kDa as determined by standard protein sizing manipulations such as polyacrylamide gel electrophoresis and centrifugation sedimentation. Additionally preferred are those polypeptides that are substantially homologous to the SYNGAP sequence shown in SEQ ID NO: 9 (ie. at least 70%, 80%, 90%, 95% up to about 99% homologous) as determined by the methods described earlier.

Particularly preferred are those polypeptides having from between about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, up to about 1500 amino acids.

The term " amino acid sequence" as used herein generally refers to any polymer preferably consisting essentially of any of the 20 naturally occurring amino acids regardless of its size. Although the term "protein" is often used in reference to relatively large proteins, and "polypeptide" or "peptide" is often used in reference to small amino acid sequences, use of these terms in this field often overlaps. Thus, it will be understood that the term generally refers to proteins, polypeptides, and peptides unless otherwise noted.

As noted, the present invention provides a variety of methods for detecting a Ras-activated signaling pathway or a phospholipid-activated pathway (sometimes called a Ras-activated or a phospholipid-activated second messenger pathway, respectively). Additionally, the present methods provide means of detecting modulation of Ras-Raf (MAP kinase) cascades. In preferred embodiments the methods include a Ras- or inositol triphosphate response system which systems can be provided in a suitable host cell or cell lysate.

In general, the response systems can be obtained by coupling a mammalian SYNGAP or a suitable fragment thereof encoded by an isolated cDNA molecule to an appropriate second messenger response system. Particular SYNGAP fragments of interest include those that modulate Ras activity or include the C2 domain as disclosed herein. The system can include but is not limited to systems relating to adenylate cyclase, phosphoinositide hydrolysis, guanylate cyclase, MAP kinase, and certain synaptic receptor proteins such as the NMDA receptor. One way to obtain a suitable response system is by transfection of an appropriate isolated polynucleotide of this invention into a suitable host cell that includes the desired second messenger system. Alternatively, a lysate can be prepared from the cell that includes purified or partially purified molecules in the pathway. When cells are used, the cells can be obtained from pre-existing cell lines or

can be generated by inserting suitable components of the desired second messenger system into those existing cell lines. Such a transfection system will preferably provide, in a single cell line, a complete Ras- or inositol triphosphate response system for detection of compounds capable of modulating the desired signalin pathway.

5

As noted, the present also embraces immune system molecules and particularly antibodies and antigen-binding fragments thereof that binding mammalian SYNGAP and particularly the SYNGAP sequence shown in SEQ ID NO: 3.

10

In particular, antibodies of the invention can be prepared by techniques generally known in the art, and are typically generated to a purified sample of SYNGAP, SYNGAP-related polypeptide. The antibodies also can be generated from an immunogenic peptide that comprises one or more epitopes of SYNGAP. Examples of such immunogenic peptides are described more fully below in the examples. As discussed above, monoclonal antibodies are sometimes preferred, although polyclonal antibodies also can be employed.

15

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More particularly, antibodies can be prepared by immunizing a mammal with a purified SYNGAP or preferably an immunogenic peptide thereof such as those peptides specifically described below. The SYNGAP or SYNGAP peptide can be administered to the mammal alone or complexed with a carrier. Suitable mammals include typical laboratory animals such as sheep, goats, rabbits, guinea pigs, rats and mice. Rats and mice, especially mice, are preferred for obtaining monoclonal antibodies. The antigen can be administered to the mammal by any of a number of suitable routes such as subcutaneous, intraperitoneal, intravenous, intramuscular or intracutaneous injection. The optimal immunizing interval, immunizing dose, etc. can vary within relatively wide ranges and can be determined empirically based on this disclosure. Typical procedures involve injection of the antigen several times over a number of months. Antibodies are collected from serum of the immunized animal by standard techniques and screened to find antibodies specific for the SYNGAP. Monoclonal antibodies can be produced in

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cells which produce antibodies and those cells used to generate monoclonal antibodies by using standard fusion techniques for forming hybridoma cells. See G. Kohler, et al., *Nature*, 256:456 (1975). Typically this involves fusing an antibody producing cell with an immortal cell line such as a myeloma cell to produce the hybrid cell. Alternatively,
5 monoclonal antibodies can be produced from cells by the method of Huse, et al., *Science*, 256:1275 (1989).

One suitable protocol provides for intraperitoneal immunization of a mouse with a composition comprising purified immunogenic SYNGAP peptide such as those
10 specifically disclosed below conducted over a period of about two to seven months. Spleen cells then can be removed from the immunized mouse. Sera from the immunized mouse is assayed for titers of antibodies specific for the immunogenic SYNGAP peptide prior to excision of spleen cells. The excised mouse spleen cells are then fused to an appropriate homogenic or heterogenic (preferably homogenic) lymphoid cell line having
15 a marker such as hypoxanthine-guanine phosphoribosyltransferase deficiency (HGPRT⁻) or thymidine kinase deficiency (TK⁻). Preferably a myeloma cell is employed as the lymphoid cell line. Myeloma cells and spleen cells are mixed together, e.g. at a ratio of about 1 to 4 myeloma cells to spleen cells. The cells can be fused by the polyethylene glycol (PEG) method. See G. Kohler, et al., *Nature, supra*. The thus cloned hybridoma
20 is grown in a culture medium, e.g. RPMI-1640. See G. E. More, et al., *Journal of American Medical Association*, 199:549 (1967). Hybridomas, grown after the fusion procedure, are screened such as by radioimmunoassay or enzyme immunoassay for secretion of antibodies that bind specifically to the purified SYNGAP or the immunogenic SYNGAP polypeptide. Preferably an ELISA is employed for the screen.
25 Hybridomas that show positive results upon such screening can be expanded and cloned by limiting dilution method. Further screens are preferably performed to select antibodies that can bind to SYNGAP or the immunogenic SYNGAP peptide in solution as well as in a mammalian fluid sample such as those obtained from a human. The isolated antibodies can be further purified by any suitable immunological technique
30 including affinity chromatography.

For human therapeutic applications, it may be desirable to produce chimeric antibody derivatives, e.g. antibody molecules that combine a non-human animal variable region and a human constant region, to thereby render the antibodies less immunogenic in a human subject than the corresponding non-chimeric antibody. A variety of types of such chimeric antibodies can be prepared, including e.g. by producing human variable region chimeras, in which parts of the variable regions, especially conserved regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. See also discussions of humanized chimeric antibodies and methods of producing same in S.L. Morrison, *Science*, 229:1202-1207 (1985); Oi et al., *BioTechniques*, 4:214 (1986); Teng et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80:7308-7312 (1983); Kozbor et al., *Immunology Today*, 4:7279 (1983); Olsson et al., *Meth. Enzymol.*, 9:3-16 (1982). Additionally, transgenic mice can be employed. For example, transgenic mice carrying human antibody repertoires have been created which can be immunized with SYNGAP or the immunogenic SYNGAP peptide. Splenocytes from such immunized transgenic mice can then be used to create hybridomas that secrete human monoclonal antibodies that specifically react with the SYNGAP or immunogenic SYNGAP peptide. See N. Lonberg et al., *Nature*, 368:856-859 (1994); L.L. Green et al., *Nature Genet.*, 7:13-21 (1994); S.L. Morrison, *Proc. Natl. Acad. Sci. U.S.A.*, 81:6851-6855 (1994).

As described previously, an antibody of this invention (polyclonal or monoclonal) or antigen-binding fragment thereof can be specifically bound to SYNGAP, e.g., in an excitatory chemical synapse. Thus, a pharmaceutical composition containing the antibody and particularly a monoclonal antibody in a pharmaceutically acceptable carrier or diluent is useful for the detection or diagnosis of cells comprising SYNGAP, e.g., neurons and other cells which may use SYNGAP as a marker such as tumors derived from neurons.

To facilitate detection or diagnosis, the desired antibody will usually be detectably-labeled. A wide spectrum of labels are known in the field such as those that

can be readily detected following administration to a subject and particularly a human patient. However, a radioisotope can be desirably used as a label because such labels are usually well suited to use in diagnostic imaging, especially those methods using scintigraphy or related methods. Choice of label will depend on several parameters the half-life or particle desired and the purpose of the detection or diagnosis. In many cases, it will be useful to choose a radionuclide that can be stored in a target tissue such as ⁹⁹Tc and other suitable radionuclides.

For *in vivo* administration, nearly any kind of carrier or diluent, preferably a pharmaceutically acceptable carrier or diluent is acceptable and can be used for preparation of the pharmaceutical composition of the present invention. It is also possible to use a plurality of carriers or diluents in combination. It is desirable to use a sterile and aqueous isotonic suspension or solution including, for example, a physiological saline and a phosphate buffer physiological saline. The pharmaceutical composition of the present invention can be administered by non-oral administration such as subcutaneous administration, intramuscular administration, intravenous administration, intraperitoneal administration, or other routes as discussed above. Preferred amounts of the pharmaceutical composition to be administered include those specific amounts discussed above.

Additionally, suitably labelled antibodies and antigen-binding fragments that bind SYNGAP or SYNGAP-related molecules can be used *in vitro*, e.g., in tissue culture, to detect excitatory chemical synapses in developing or established neuronal cell cultures. In this example, the antibody can be detectably-labeled with a variety of labels known in the field.

By the term "specific binding" or similar term is meant a molecule disclosed herein which binds another molecule, thereby forming a specific binding pair, but which does not recognize and bind to other molecules as determined by, e.g., Western blotting,

ELISA, RIA, gel mobility shift assay, enzyme immunoassay, competitive assays, saturation assays or other suitable protein binding assays known in the field.

As specifically shown in the examples which follow, we have isolated a novel
5 Ras-GTPase activating protein called SYNGAP. The SYNGAP protein specifically
interacts with the PDZ domains of PSD-95 and SAP102 *in vitro* and *in vivo*. SYNGAP is
selectively expressed in brain and is highly enriched at excitatory synapses where it is
present in a large macromolecular complex with PSD-95 and the NMDA receptor.
SYNGAP stimulates the GTPase activity of Ras suggesting that it negatively regulates
10 Ras activity at excitatory synapses. Ras signaling at the postsynaptic membrane may be
involved in the modulation of excitatory synaptic transmission by NMDA receptors and
neurotrophins. These results indicate that SYNGAP may play an important role in the
modulation of synaptic plasticity.

15 In particular, the examples below show that SYNGAP is a brain-specific protein
of about 125 kDa that is associated in a large complex with PSD-95/SAP90, SAP102, and
the NMDA receptor in brain. Immunocytochemical studies show that SYNGAP is highly
enriched at excitatory synapses and colocalizes with PSD-95/SAP90, SAP102, and the
NMDA receptor. The GTPase activating domain shares significant homology with other
20 rasGAPs and has rasGAP activity. These results suggest that the PSD-95/SAP90 family,
through SYNGAP, may play a role in the regulation of synaptic ras signal transduction
cascades.

The following non-limiting examples are illustrative of the present invention.
25

The following Examples 1-8 refer to the isolation and characterization of
SYNGAP-c (GAPSYN) unless otherwise specified.

30 EXAMPLE 1- Molecular Characterization of SYNGAP

Novel proteins that interact with the PDZ domains of the PSD-95/SAP90 family of proteins were identified by using the third PDZ domain of SAP102 to screen a yeast two-hybrid hippocampal cDNA library (Fields, S. and Song, O. (1989) *Nature*. 340:245; Dong, H., et al., (1997) *Nature*. 386:279). Screening two million clones led to the isolation of a single clone with an open reading frame of 168 amino acids. Successive screening of a λ ZAP hippocampal cDNA library yielded several full length cDNAs with insert sizes of ~7.4 kb. The full length cDNA had an open reading frame encoding 1135 amino acids with a calculated molecular weight of 124.7 kDa. See Figures 3A- D; and Figures 4A-B.

Analysis of the amino acid sequence of the protein indicates that it is novel and contains several functional domains (Figure 4B) . The amino terminal half of the protein includes a region that has some homology to PH (pleckstrin homology) domains, a C2 domain that may be involved in the binding of Ca^{2+} and phospholipid, and a rasGAP domain. The C-terminal half includes a repeat of 10 histidines which may be involved in metal chelation, several potential serine and tyrosine phosphorylation sites and a T/SXV motif (QTRV) required for the interaction with SAP102 and PSD-95 (see below). The amino acid sequence also predicts that it is a cytosolic protein that has no apparent transmembrane region and no signal peptide. Alignment of the GAP domain of SYNGAP with other ras-GAPs (Figure 1C) indicates that the amino acids are critical for the interaction of rasGAPs with Ras and the stimulation of Ras GTPase activity are conserved (Scheffzek, K., et al. (1997) *Science*. 277:333). Because of its ras-GTPase activity and selective localization at excitatory synapses (see below), this protein was named SYNGAP.

Figures 4A-C are more particularly described as follows: Figure 4A: amino acid sequence of SYNGAP (SEQ ID NO. 9). SYNGAP is a 1135 amino acid protein that contains a ras-GAP domain (shaded) and several regulatory domains. Consensus serine and tyrosine phosphorylation sites are underlined. Figure 4B: In addition to the ras-GAP

domain (GAP) the structure of SYNGAP includes a domain that shares a partial
homology to PH domains (PH) followed by a C2 domain (C2). The C-terminal four
amino acids (QTRV) required for binding to PDZ domains are indicated. Figure 4C:
alignment of the ras-GAP domain of SYNGAP with *Rattus norvegicus* (rn) ras-GAP
(L13151) and *Homo sapiens* neurofibromin (hs 1NF1, M38107). Identical residues are in
bold type. The residues that participate in catalysis (*) and in the interaction with Ras (+)
are indicated.

1. Identification and Cloning of SYNGAP

The yeast two-hybrid system was utilized to find protein(s) that interact with the
third PDZ domain of SAP102. The third PDZ domain (amino acids 367 to 452) was
generated by PCR using a pair of oligonucleotides with restriction digestion sites for *Sal* I
and *Bgl* II sense (5'-ACGCGTCGACCAGAGAGCCCCGCAAG-3' (SEQ ID NO. 22))
and antisense (5'-GAAGATCTAGGTCTATACTGGGCCAC-3' (SEQ ID NO. 23)) and
was subcloned into the pPC97 yeast vector containing the GAL4 DNA binding domain
(Chevray, P. M., and Nathans, D. (1992) *Proc. Natl. Acad. Sci. USA.* 89:5789). The bait
plasmid was then transformed into Y190 yeast cells (Durfee, T., et al. (1993) *Genes Dev.*
7:555; Staudinger, J., et al. (1995) *J. Cell Biol.* 128:263) and a two-hybrid screening was
performed using a random-primed cDNA library from rat hippocampus subcloned into
the *Sal* I /*Not* I site of the pPC86 vector containing the GAL 4 transcription activation
domain (Brakeman, P. R., et al. (1997) *Nature.* 386:284; Dong et al., *supra*). Positive
clones were selected on plates lacking leucine, tryptophan, and histidine with 50 mM
3-aminotriazole and confirmed by filter assay for β -galactosidase activity (Breedon, L.,
and Nasmyth, K. (1985) *Cold Spring Harb. Symp. Quant. Biol.* 50: 643). For cloning of
the full length of SYNGAP, successive rounds of phage library screening were performed
with rat hippocampal λ ZAP cDNA libraries (dT-primed and random-primed). The
nucleic acid sequence of the SAP102 protein can be found in Mueller, et al. (1996)
Neuron. 17:255. the hippocampal λ ZAP cDNA library was made according to standard
methods. See e.g., Ausubel et al. *supra* and Sanbrook et al., *supra*.

1. Transfection of HEK 293T Cells

SYNGAP subcloned into pGW-1 mammalian expression vector (10 µg) was transfected into HEK 293T cells in 10 cm culture dishes by calcium phosphate co-precipitation (Blackstone, C. D., et. al. (1992) *J. Neurochem.* 58:1118). After 48 hours of transfection the cells were harvested and analyzed by SDS-PAGE and immunoblotting.

EXAMPLE 2- Interaction of SYNGAP and PDZ domains Inside Cells

The interaction of SYNGAP with the PDZ domains of PSD-95/SAP90 and SAP102 was further studied in the yeast two-hybrid system. As shown in Table 1 below, SYNGAP interacts with all of the PDZ domains of PSD-95/SAP90. Deletion analysis of SYNGAP's C-terminus revealed that the C-terminal -TRV is critical for binding of SYNGAP to the PDZ domains (Table 1). In addition, the last valine residue is essential for interaction, as has been shown with other ligands for the PSD95/SAP90 family of proteins.

1. Yeast Interaction Studies

The yeast two-hybrid system was used to check for interaction of the carboxy terminal tail of SYNGAP with the various PDZ domains of SAP102 and PSD-95/SAP90. The PDZ domains of PSD-95/SAP90 and SAP102 were amplified by PCR and subcloned into the yeast vector, pPC 86. PDZ domain 1 of PSD-95 covers the amino acids from 40 to 160; PDZ domain 2, from 156 to 248; and PDZ domain 3, from 298 to 403. See e.g., Cho et al., (1992) *Neuron* 9:929 for disclosure relating to the PSD-95 sequence.

The original yeast clone with the intact C-terminal tail of SYNGAP (968- 1136) was subcloned into pPC97 and was used to check for interaction with the various PDZ domains. The requirement of the C-terminal T/SXV motif was investigated by subcloning PCR generated deletion mutants of SYNGAP (894 to 1134 for -QTR* and 894 to 1132 for -Q*). The yeast vectors are transformed into Y190 and scored by growth without

leucine, tryptophan, and histidine, in 100 mM 3-aminotriazole and filter assay for β -galactosidase activity.

Table 1 PDZ Domain Interaction with the Carboxy
Terminal Tail Motif, -QTRV, of SYNGAP

	(-QTRV*)		(-QTR*)		(-Q*)	
	HIS3	B-Gal	HIS3	B-Gal	HIS3	B-Gal
SAP102						
PDZ 3	+	+	-	-	-	-
PSD-95						
PDZ 1	+	+				
PDZ 2	+	+				
PDZ 3	+	+				

EXAMPLE 3- Brain-Specific Expression of SYNGAP mRNA and Protein

Northern blotting with the *Sal* I / *Not* I fragment of the yeast clone revealed a mRNA of approximately 7 kb which was detected only in brain and was expressed predominantly in cortex, hippocampus, and olfactory bulb (Figure 7A). In addition, a less abundant mRNA of about 9 kb was observed in these same tissues. To characterize the SYNGAP protein, an anti-SYNGAP antibody was generated against its last C-terminal 20 amino acids (Figure 7B). The anti-SYNGAP C-terminal antibody specifically recognized 130 kDa SYNGAP protein when it was expressed in HEK 293 cells. In brain, the anti-SYNGAP antibody recognized a doublet or triplet of proteins at 130 kDa (Figure 7C). Preabsorption of the anti-SYNGAP antibody with the peptide immunogen completely eliminated the recognition of the 130 kDa protein confirming the specificity of the antibody. The origin of the triplet in brain is not entirely understood. Without wishing to be bound to any specific theory, the triplet may arise from alternative splicing

or posttranslational modification. Using the anti-SYNGAP antibody, the expression of SYNGAP in different tissues was examined. As with the Northern blot data, SYNGAP protein was found to be expressed exclusively in brain with high levels in the cortex and hippocampus and relatively low levels in cerebellum (Figure 7C). SYNGAP was found to
5 be selectively localized to membrane fractions and was resistant to Triton X-100. CHAPS. and RIPA extractions from synaptic plasma membranes (Figure 7D) similar to other proteins associated with the PSD such as PSD-95/SAP90, SAP102, and the NMDA receptor (Cho, K.O., et al. (1992) *Neuron*. 9:929; Lau et al., *supra* 1996; Kim, E., et al. (1997) *J. Cell. Biol.* 136:669).

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Figures 7A- 7D are more specifically described as follows: Figure 7A Northern blot of SYNGAP mRNA shows high level of SYNGAP mRNA expression in brain. Figure 7B: antibodies were generated against SYNGAP and used to identify SYNGAP in rat tissues using immunoblot techniques. These antibodies recognized a 130kDa protein
15 in HEK293T cells transfected with the SYNGAP cDNA (Lane 2) and in rat brain homogenates (Lane 3). The 130kDa protein was not detected in mock transfected HEK293T cells (lane 1) and immunorecognition of the 130kDa protein in brain was blocked by preabsorbtion of the antibody with the antigen (Lane 4). Figure 7C: immunoblots of rat tissues with the SYNGAP antibody demonstrates that the expression
20 of the SYNGAP protein is brain specific. Figure 7D: immunoblots of rat brain cortex crude homogenates (cortex) and cytosolic and membrane fractions demonstrate that SYNGAP is membrane-associated. Cortical membranes were extracted with the indicated detergents and then separated into soluble (S) and pellet fractions (P) and the distribution of SYNGAP detected using immunoblot techniques. SYNGAP is resistant to membrane
25 extraction with non-denaturing detergents similar to other proteins located in the PSD fraction.

1. RNA Preparation and Northern Blot Analysis

Total RNA from various tissues of male Sprague Dawley rat at P14 was isolated
30 with RNazol (Tel-Test) according to the manufacturer's protocol. The RNA (10 µg per

lane) was separated on 1.2% formaldehyde agarose gel, transferred onto GeneScreen Plus membrane (Du Pont NEN) and then hybridized with a [α - 32 P]-dCTP labeled *Sal* I /*Not* I fragment of the original yeast clone. The result was visualized using a phosphoimager cassette. The blot was stripped and rehybridized with radioactively labeled GAPDH
5 (*Hind* III/*Bam* H I fragment) DNA to ensure equal loading of RNA per lane.

EXAMPLE 4- SYNGAP Associates with PSD-95 and SAP102 *in vivo*

Interaction of SYNGAP, PSD-95/SAP90 and SAP102 was examined in the brain
10 *in vivo*. As shown in Figure 8, immunoprecipitation of PSD-95/SAP90 or SAP102 from deoxycholate solubilized brain membrane preparations resulted in the specific coimmunoprecipitation of SYNGAP. Moreover, using antibodies to the NR1 subunit of the NMDA receptor, SYNGAP was also found to be specifically associated with NMDA receptors (Figure 8). Preabsorption of the SAP102 and NR1 antibodies with their antigens
15 blocked the coimmunoprecipitation of SYNGAP confirming the specificity of the association. In contrast, SYNGAP did not coimmunoprecipitate with the NMDA receptor GluR1 subunit. These results strongly suggest that SYNGAP was associated with an NMDA receptor complex containing NMDA receptor subunits and PSD-95/SAP90 family members.

20 Figure 8 is more specifically described as follows: Synaptic plasma membranes were solubilized with deoxycholate and the resulting detergent extract (input) was used to immunoprecipitate the indicated proteins (IP). The immunoprecipitates were then analyzed for the presence of SYNGAP, NMDAR1, PSD-95 and GluR1 by immunoblot
25 techniques as indicated above.

1. Brain Membrane Preparation and Solubilization

Membrane preparations (P2) and solubilization was carried out according to the procedures described by Luo et al. (1997) with modifications. Cortex and hippocampus
30 from a male Sprague-Dawley rat, age of 4 to 6 weeks were homogenized twice using a

glass-Teflon homogenizer with protease inhibitors (antipain, chymotrypsin, leupeptin, Trasylol, 0.1 mM PMSF). After determining the protein concentration of the P2 fraction by a Coomassie assay (Pierce), aliquots of the proteins at 3 mg/ml were stored at -80°C until use. For coimmunoprecipitation, PA (300 µg per IP) was solubilized by 1.0 % sodium deoxycholate followed by 0.1 % Triton X-100 and the preparation was centrifuged for 10 minutes at 100,000 x g. The supernatant was then used for coimmunoprecipitation.

For detergent extraction of SYNGAP, synaptic plasma membranes were prepared and solubilized using various detergents, SDS, Triton X-100, CHAPS, and RIPA, according to the procedure described by Lau et al. *supra*.

EXAMPLE 5- Detection of SYNGAP at Specific Synapses

The subcellular distribution of SYNGAP in neurons was examined in low density hippocampal cultures using the anti-SYNGAP antibody. SYNGAP was expressed in the cell body of neurons but the pattern of immunoreactivity was strikingly punctuate along the processes of the neurons, suggesting that SYNGAP may be localized to synapses (Figure 9A). Double labeling the hippocampal neurons with antibodies against SYNGAP and the synaptic marker protein synaptophysin demonstrated that SYNGAP is specifically localized to synapses (Figures 9B- 9D). The staining of the neurons with the SYNGAP antibody was blocked by preabsorption of the antibody with the antigenic peptide confirming the specificity of the antibody (Figures 9E-9F).

As noted above in Example 3, SYNGAP associated with PSD-95 and SAP102 *in vivo*. Next, the colocalization of the PSD95/SAP90 family members with SYNGAP was examined. It was found that SYNGAP colocalized with PSD-95 (Figure 10A-10I). This observation provides additional support for SYNGAP association with PSD-95.

To determine whether SYNGAP is localized to both excitatory and inhibitory synapses, neurons were double labeled with SYNGAP antibodies and antibodies against the NR1 subunit of the NMDA receptor to label excitatory synapses or anti-glutamic acid decarboxylase (GAD) antibodies to label inhibitory synapses. As shown in Figure 5B SYNGAP colocalized with NMDA receptors and was present at all excitatory synapses. In contrast, SYNGAP was not observed at GABAergic synapses (Figure 10G-10I).

Figures 9A- 9F are more particularly explained as follows: Figure 9A: low density hippocampal cultures were stained with the SYNGAP antibody and visualized with a Rhodamine-coupled secondary antibody. SYNGAP is present in the cell soma and is clustered on the neuronal processes. Figures 9B-9D: double labeling of the hippocampal cultures with antibodies against SYNGAP and the synaptic marker synaptophysin demonstrates that SYNGAP is synaptically localized. Figures 9E-9F: the staining of the hippocampal neurons with the SYNGAP antibody is specifically blocked by preincubation of the antibody with the antigen. The right panel shows that the double labeling with anti-synaptophysin antibody is not blocked by the SYNGAP antigen.

Figures 10A -10I are also more particularly described as follows: Figure 10A-10C: double labeling of hippocampal neurons with antibodies against SYNGAP and PSD-95 demonstrates that SYNGAP and PSD-95 are colocalized in the neurons. Figure 10D-10F: SYNGAP exists exclusively at excitatory synapses as shown by the colocalization of SYNGAP with the NR1 subunit of the NMDA receptor. Figures 10G-10I: to exclude the possibility that SYNGAP is found at inhibitory synapses, the hippocampal neurons were double labeled with the antibodies to SYNGAP and the GABAergic synaptic marker GAD. GAD staining does not overlap with the expression pattern of SYNGAP.

EXAMPLE 6- SYNGAP has Ras-GAP Activity

SYNGAP shows homology with the GAP domain of rasGAPs suggesting that SYNGAP may regulate the GTPase activity of Ras (Boguski and McCormick, (1993) Nature 366: 643). A glutathione S-transferase (GST) fusion protein of the rasGAP domain of SYNGAP was made and incubated with a purified H-ras GST fusion protein in the presence of [γ - 32 P]-GTP. It was found that GST-ras fusion proteins exhibited a low intrinsic GAP activity in the absence of a rasGAP (Figure 11). In contrast, addition of the GST fusion protein of the GAP domain of SYNGAP dramatically stimulated the GTPase activity of Ras (Figure 11). Control GST-backbone protein (Figure 11) or a GST-SAP102 fusion protein had no effect on ras-GTPase activity.

1. Fusion Protein Construction and Preparation

The first 119 amino acids of SAP102 was amplified by PCR and subcloned in frame into pTrcHisB vector (Invitrogen) via the *Bam*H I and *Eco*R I restriction digest sites. The construct was then transformed into BL21 bacteria and following an induction of expression with IPTG. The protein was purified in a denaturing condition according to the protocol provided by the QIA-expressionist (Qiagen). The GAP domain (266-521) subcloned into pGEX-4T2 and a GST-Ras fusion protein was obtained and expressed in BL21 cells. The fusion protein was purified using glutathione coupled agarose. All of the above proteins were analyzed by SDS-PAGE followed by Coomassie Blue Staining.

2. GAP Assay

The GAP assay was performed following the methods described by Settleman et al. (1992) *supra* with modifications. Briefly, 0.5 to 1 μ M of GST-Ras was incubated with 20 nM [γ - 32 P]-GTP (6,000 Ci/mmol, Du Pont-NEN) in 50 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mg/ml BSA, and 1 mM DTT for 10 minutes at room temp. GST-GAP or control GST fusion protein (200 ng) was then added in 50 mM Tris, pH 7.5, 5 mM MgCl₂, 1 mM DTT and incubated for the indicated times. After stopping the reaction (by adding 5 volumes of ice-cold 50 mM Tris, pH 7.5, 5 mM MgCl₂; 1 mM

DTT), the mixture was filtered through HA membranes (Millipore) and the filters were counted for ^{32}P in scintillation cocktail.

Results of the GAP assay are shown in Figure 11. Time course of the GTPase activity of GST H-Ras fusion protein in the presence and absence of the GAP domain of SYNGAP. Incubation of a GST-ras fusion protein with a control GST fusion protein (--◇--) had little effect on the slow intrinsic GTPase activity of GST-Ras. In contrast, addition of the GST-fusion protein containing the GAP domain of SYNGAP dramatically stimulated the GTPase activity of GST-Ras (-□-).

EXAMPLE 7- Generation and Purification of SYNGAP and SAP102 Antibodies

The last 20 amino acids of SYNGAP (KRLLEDAQRGSFPPWVQQTRV (SEQ ID NO. 21) were synthesized and purified. The protocol for generation and purification of polyclonal antibody was described by Blackstone et al. (Blackstone et al., 1992 *supra*). Briefly, the peptide was crosslinked to thyroglobulin with glutaraldehyde and injected into New Zealand white rabbits to generate antiserum (Covance). The antiserum was then purified with Affi-Gel 10 resin (Bio-Rad) coupled to BSA covalently bound to the peptides (Lau et al., (1996), *supra*). The N-terminal SAP102 fusion protein with hexahistidine tag was immunized similarly to generate a polyclonal rabbit antibody and the antiserum was purified using the antigen coupled to Affi-Gel 10.

1. Coimmunoprecipitation and Immunoblotting

To affinity purified antibodies, about 1 to 2 μg were preincubated with 50 μl of 1:1 slurry of protein A-Sepharose for 1 hour and the protein A-antibody complex was spun down at 2000 rpm for 2 minutes. The clarified supernatant of solubilized P2 fraction was then added to the sepharose beads and the mixture incubated for 2 to 3 hours at 4° C. The mixture was washed once with 1% Triton X-100 in immunoprecipitation buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4 , 1.4 mM KH_2PO_4 , 5 mM EDTA, 5 mM EGTA, 1 mM sodium vanadate. 10 mM sodium pyrophosphate, 50 mM NaF, 20 units/ml

Trasylol and 0.1 mM phenylmethylsulfonyl fluoride), twice with 1 % Triton X-100 in immunoprecipitation buffer plus 300 mM NaCl, and three times with immunoprecipitation buffer. The proteins were eluted by Laemmli sample buffer (Laemmli, 1970) and were separated by SDS-PAGE. The gel was transferred to a PVDF (Millipore) membrane and the membrane was blocked and immunoblotted as described by Lau et al. (1996), *supra*. When the antibodies were blocked with antigens, they were preincubated with the peptide at concentration of 100 µg/ml or fusion proteins at concentration of 50 µg/ml. The antibodies used in the experiments have been previously described: anti-NR1a C-terminal antibody (Tingley, W. G., et al. (1993) *Nature*. 364:70), anti-GluR1 C-terminal antibody (Blackstone et al., 1990), anti-NR2B C-terminal antibody (Lau, L. F., and Huganir, R. L. (1995) *J. Biol. Chem.* 270:20036), and anti-PSD-95 antibody (Gift from Dr. J. S. Trimmer)

2. Immunocytochemistry

Low density hippocampal neuronal culture was performed following the procedure described by Goslin and Banker (Goslin and Banker, 1991). After 13 to 90 days in culture, the neurons were fixed and stained with affinity purified antibodies as previously described with minor modifications (Lau and Huganir, 1995 *supra*; O'Brien, R. J., et al. (1997) *J. Neurosci.* 17:7339; Rao, A. and Craig, M. (1997) *Neuron*. 19:801).

Examples 1 to 8 show the isolation and characterization SYNGAP, a novel synaptic rasGAP that directly interacts with the PSD-95/SAP90 family of proteins. SYNGAP is highly expressed in brain, is not detected in other tissues and is especially abundant in the cortex, hippocampus, and olfactory bulb. SYNGAP is found exclusively at excitatory synapses in hippocampal neurons in culture. The SYNGAP protein is tightly associated with synaptic membranes, and exists as two or more alternate forms. Due to its synaptic localization and association with the PSD it is uniquely positioned to regulate signal transduction events that may involve ras signaling pathways at excitatory synapses.

EXAMPLE 8- Isolation of Human SYNGAP

It is possible to isolate *Homo sapiens* SYNGAP in accord with the present disclosure. For example, in one approach, the SYNGAP can be obtained by screening a
5 human brain cDNA γ ZAP phage library (Stratagene) at high stringency conditions. More particularly, the hybridization can be carried out by incubation at 65°C overnight in 0.08 Na phosphate, pH 6.8, 0.05 mNa citrate, 0.5 mNaCl, 5X Denhardt's solution, 0.5% SDS, 0.1 mg/ml salmon sperm DNA with a probe concentration of about 0.4 million CPM/ml. A preferred probe can be made by standard methods from any of the *Rattus norvegicus*
10 SYNGAP DNA sequences shown in SEQ ID Nos. 1-2, 4-5, or 7-8. Especially preferred probes have a length between about 500 to about 2000 bases. Such probes are typically restriction fragments that can be readily obtained by restriction enzyme digestion of a desired SYNGAP cDNA followed by purification of the fragment. Typically, the fragment is detectably-labeled, e.g., with 32 P to facilitate screening. The homology of the
15 clone isolated between the two different species (i.e. rat and human) can be compared: an identity of 70 to 80% may be observed at the DNA sequence level. At the protein, about 70 to about 90% identity may be observed across a substantial length of the human gene. The isolated human clone can be analyzed in accordance with the SYNGAP sequences disclosed herein to identify mRNA expression pattern, GAP activity, and/or participation
20 in a MAP kinase cascade.

The examples also show that SYNGAP has an organized and domain structure which is involved in the regulation of its GAP activity. In addition to the ras-GAP domain the amino terminus contains a region that is partially homologous to pleckstrin
25 homology (PH) domains, a phospholipid binding module of about 100 amino acids that binds polyphosphatidylinositides which is thought to serve as a signal dependent membrane adaptor (Shaw, G. (1996) *Bioessays*. 18:35). The N-terminal region also contains a C2 domain which has been shown to be involved in the binding of Ca^{2+} in a phospholipid-dependent manner (Luo, J. H., and Weinstein, I. B. (1993) *J. Biol. Chem.*
30 268:23580) in protein kinase C, synaptotagmin and rabphilin-3A (Nishizuka, Y. (1988)

Nature. 334:661; Perin, M. S., et al. (1991) *J. Biol. Chem.* 266:623; Yamaguchi, et al., (1993) *J. Biol. Chem.* 268:27164). C2 domains have also been found in other rasGAPs such as human p120GAP and GAP1^{IP4BP} (Cullen, P.J., et al. (1995) *Nature*. 376:527). The presence of these two domains suggests that SYNGAP may respond to changes in Ca²⁺ and phospholipid second messengers. The association of SYNGAP with the NMDA receptor complex suggests that SYNGAP may specifically respond to changes in Ca²⁺ mediated by activation of NMDA receptors. The C-terminal half of SYNGAP also contains potential regulatory domains including many consensus phosphorylation sites for CaMKII and protein tyrosine kinases. CaMKII is very abundant in the postsynaptic density and has been implicated as having a key role in the modulation of synaptic plasticity (Nicoll, R.A. and Malenka, R.C. (1995) *Nature*. 377:115). In addition, SYNGAP has ten consecutive histidine residues which may bind divalent transition metals such as Zn²⁺ and Cu²⁺ and play some role in the regulation of SYNGAP function.

The *in vivo* association of SYNGAP with the NMDA receptor complex and PSD-95 and SAP 102 suggests that SYNGAP plays a specific role in the modulation of ras signaling at excitatory synapses. The specific localization of SYNGAP to excitatory synapses suggests that SYNGAP may play a critical role in the regulation of BDNF signaling in the postsynaptic membrane.

The identification of SYNGAP as a PSD-95 associated protein provides the first evidence that the PSD-95/SAP90 family forms a complex with signal transduction molecules involved in the ras pathway and suggest that ras plays specific roles in the regulation of excitatory synaptic transmission at the postsynaptic membrane. SYNGAP may also regulate ras signaling in response to Ca²⁺.

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modification and improvements within the spirit and scope of the invention as set forth in the following claims.

[illegible]

What is claimed is:

1. An isolated polynucleotide encoding a mammalian Synaptic GTPase Activating Protein (SYNGAP).
2. The polynucleotide of claim 1, wherein the polynucleotide comprises the sequence of any one of SEQ ID Nos. 1-2, 4-5, or 7-8.
3. The polynucleotide of claim 1, wherein the mammalian SYNGAP has a molecular weight of between about 100 kDa to about 140 kDa as determined by polyacrylamide gel electrophoresis.
4. The polynucleotide of claim 1, wherein the polynucleotide has at least about 70 percent sequence identity to any one of SEQ ID Nos. 1-2, 4-5, or 7-8.
5. The polynucleotide of claim 1 wherein the polynucleotide is cDNA or RNA.
6. An isolated polynucleotide that is capable of hybridizing to any one of the sequences shown in SEQ ID Nos. 1-2, 4-5, or 7-8 under moderate stringency conditions.
7. The isolated polynucleotide of claim 6, wherein the polynucleotide hybridizes to any one of the sequences shown in SEQ ID Nos. 1-2, 4-5, or 7-8 under high stringency conditions.
8. The polynucleotide of claim 7, wherein the polynucleotide is between about 12 to about 50 nucleotides in length.
9. The polynucleotide of claim 7, wherein the polynucleotide is between about 100 to about 3500 nucleotides in length.

10. The polynucleotide of claim 9, wherein the polynucleotide encodes an amino acid sequence that is capable of activating Ras GTPase by at least about 20% in a standard Ras GTPase assay.

11. The polynucleotide of claim 10, wherein the polynucleotide encodes an amino acid sequence capable of binding a PDZ domain as determined by a standard PDZ domain binding assay.

12. The polynucleotide of claim 11, wherein the polynucleotide encodes an amino acid sequence capable of binding an NR1 subunit of an NMDA receptor as determined by a standard NMDA receptor binding assay.

13. The polynucleotide of claim 12, wherein the amino acid sequence encoded by the polynucleotide comprises at least a RAS GTPase activating protein (GAP) domain and a C-terminus comprising the following amino acid sequence: (T or S) X V; wherein X is an amino acid.

14. The polynucleotide of claim 13, wherein the amino acid sequence encoded by the polynucleotide further comprises a pleckstrin homology (PH) and a C2 domain.

15. The polypeptide of claim 14, wherein the amino acid sequence encoded by the polynucleotide comprises in an N- to C- terminus orientation: the pleckstrin homology (PH), the C2 domain, the GAP domain and the C-terminal amino acid sequence.

16. The polynucleotide of claim 15, wherein the polynucleotide encodes an amino acid sequence comprising in an N- to C-terminus orientation at least about the following amino acids of SEQ ID NO: 9: 4 to 72, 87 to 190, 266 to 502, and 1132 to 1135.

17. A polynucleotide encoding a mammalian SYNGAP as shown in any one of SEQ ID Nos : 3, 6 or 9; or a fragment or a derivative thereof.
18. The polynucleotide of claim 1, wherein the SYNGAP is a human or a rat SYNGAP.
19. A fragment or a derivative of the polynucleotide of claim 1.
20. A recombinant vector comprising the polynucleotide of claim 1 or 19.
21. A host cell comprising the polynucleotide of claim 20.
22. An isolated mammalian GTPase Activating Protein at Synapses (SYNGAP) having an apparent molecular weight of between about 100 to about 150 kDa as determined by polyacrylamide gel electrophoresis.
23. An isolated polypeptide having at least about 70 percent sequence identity to any one of the amino acid sequences of SEQ ID NOs:3, 6 or 9.
24. An isolated polypeptide having the sequence shown in SEQ ID NO. 3, 6 or 9 or a fragment or a derivative thereof.
25. A method for producing a mammalian GTPase Activating Protein at Synapses (SYNGAP), the method comprising culturing a host cell of claim 21 in medium under conditions suitable for expression of the SYNGAP in the host cell or medium.
26. An antibody or antigen-binding fragment thereof capable of binding the amino acid sequence shown in SEQ ID NO. 9, the binding being blocked by at least

about 90% by contact with the amino acid sequence shown in SEQ ID NO. 21, wherein the blocking is determined by an immunoprecipitation assay or a Western immunoblot.

27. The antibody of claim 26, wherein the antibody is capable of binding excitatory synapses as determined by microscopy.

28. The antibody of claim 27, wherein the antibody is a monoclonal antibody or an antigen-binding fragment thereof.

29. A kit comprising:
container means comprising at least one of: 1) an antibody capable of binding mammalian Synaptic GTPase Activating Protein (SYNGAP), 2) an isolated polynucleotide comprising sequence with at least about 70% sequence homology to any of the sequences shown in SEQ ID Nos. 1-2, 4-5, or 7-8, 3) a pair of oligonucleotide primers capable of hybridizing to the sequence shown in any one of SEQ ID Nos. 1-2, 4-5, or 7-8 under high stringency conditions; and 4) a polypeptide with at least 70% sequence homology to any one of the sequences shown in SEQ ID NO: 3, 6 or 9.

30. The kit of claim 29, wherein the container means comprises a system for: 1) treating or preventing a disorder in a mammal associated with the SYNGAP, or 2) detecting excitatory synapses in a cell or group of cells *in vitro* or *in vivo*.

31. A method for modulating excitatory synapse function in a cell or group of cells, the method comprising administering to the cells a modulation effective amount of a polynucleotide of any one of claims 1 to 20 or fragment or derivative thereof.

32. A method for modulating excitatory synapse function in a cell or group of cells, the method comprising administering to the cells a modulation effective amount of the SYNGAP of any one of claims 21 to 24 or fragment or derivative thereof.

33. The method of claim 31 or 32, wherein the modulation comprises an increase excitatory synapse number as determined by microscopy or centrifugation.

34. A method for treating a disorder associated with mammalian GTPase Activating Protein at Synapses (SYNGAP) comprising administering to a patient suffering from or susceptible to such disorder an effective amount of an isolated polynucleotide of any one of claims 1 to 20 or fragment or derivative thereof.

35. A method for treating a disorder associated with mammalian GTPase Activating Protein at Synapses (SYNGAP) comprising administering to a patient suffering from or susceptible to such disorder an effective amount of an isolated SYNGAP of any one of claims 21 to 24 or fragment or derivative thereof.

36. The method of claim 35 or 36, wherein the disorder is a neurological disorder of the central (CNS) or peripheral (PNS) nervous system.

37. The method of claim 36, wherein the CNS disorder is at least one of an affective disorder, a cognitive disorder, or a neurodegenerative disorder.

38. The method of claim 37, wherein the affective disorder is depression.

39. The method of claim 37, wherein the cognitive disorder is at least one of memory loss, a learning disability, or schizophrenia.

40. The method of claim 38, wherein the learning disability is attention deficit disorder (ADD).

41. The method of claim 37, wherein the degenerative disorder is at least one of Parkinson's disease (PD), Huntington's disease (HD), senile dementia, or Alzheimer's disease (AD).

42. The method of claim 36, wherein the PNS disorder is amyotrophic lateral sclerosis.

43. The method of claim 37, wherein the neurological disorder is associated with at least one of trauma, an immune response or ischemia.

44. A method for identifying a compound useful in the diagnosis or treatment of a disorder relating to Synaptic GTPase Activating Protein (SYNGAP), the method comprising:

- a) culturing cells capable of forming synapses comprising SYNGAP under conditions conducive to forming or maintaining synapses,
- b) contacting the cells with a candidate compound,
- c) analyzing the cells for an increase or decrease in the number of synapses; and
- d) detecting the increase or decrease as indicative of the compound useful in the diagnosis or treatment of the disorder relating to SYNGAP.

45. A method for detecting a compound capable of modulating a Ras-activated second messenger pathway, the method comprising:

- a) providing a Ras response system comprising a recombinant mammalian synaptic GTPase Activating Protein (SYNGAP),
 - b) contacting the Ras response system with a candidate compound,
 - c) analyzing the Ras response system for an increase or decrease in Ras activity;
- and

d) detecting the increase or decrease in the Ras activity as indicative of the compound capable of modulating the Ras-activated second messenger pathway.

46. The method of claim 44 or 45, wherein the Ras response system comprises Ras, adenylate cyclase and an isolated polynucleotide encoding the SYNGAP.

47. The method of any one of claims 44 to 46 wherein the Ras response system is provided in a host cell or a lysate thereof.

48. A method for detecting a compound capable of modulating a phospholipid-activated second messenger pathway, the method comprising:

a) providing an inositol triphosphate response system comprising a recombinant mammalian synaptic GTPase Activating Protein (SYNGAP),

b) contacting the inositol triphosphate response system with a candidate compound,

c) analyzing the inositol triphosphate response system for an increase or decrease in phospholipase C activity; and

d) detecting the increase or decrease in the phospholipase C activity as indicative of the compound capable of modulating the phospholipid-activated second messenger pathway.

49. The method of claim 48, wherein the phospholipase C is a phosphoinositide-specific enzyme.

50. The method of claim 48 or 49, wherein the inositol triphosphate response system further comprises phosphatidylinositol (PI), and an isolated polynucleotide encoding the SYNGAP.

51. The method of claim 50, wherein the inositol triphosphate response system further comprises at least one of diacylglycerol, protein kinase C, and inositol 1,4,5 triphosphate (INSP₃).

52. The method of claim 51, wherein the phospholipid-activated second messenger pathway is capable of at least one of calcium (Ca⁺²) release or protein phosphorylation.

53. The method of any one of claims 48 to 52, wherein the inositol triphosphate response system is provided in a host cell or a lysate thereof

54. A method for detecting a compound capable of modulating phospholipid-dependent calcium (Ca⁺²) binding to a mammalian synaptic GTPase Activating Protein (SYNGAP) C2 domain, the method comprising:

a) mixing the C2 domain with a phospholipid, calcium (Ca⁺²) and (SYNGAP) or a fragment thereof comprising the C2 domain, the mixing being under conditions conducive to forming a complex,

b) contacting the mixture with a candidate compound,

c) analyzing the mixture for formation of the complex; and

d) detecting the complex as indicative of the compound capable of modulating the phospholipid-dependent binding between the calcium (Ca^{+2}) and the C2 domain of the SYNGAP.

55. The methods of any one of claims 44 to 54, wherein the SYNGAP has a sequence represented by SEQ ID NO:3, 6 or 9; or a fragment or derivative thereof.

56. A method for detecting a test amino acid sequence capable of binding a mammalian synaptic GTPase Activating Protein (SYNGAP), the method comprising contacting the test amino acid sequence with SYNGAP or the fragment or the derivative thereof under conditions conducive to forming a complex; and detecting the complex as indicative of the test amino acid sequence capable of binding the SYNGAP.

57. The method of claim 56, wherein the detecting step comprises at least one of immunoprecipitation, affinity chromatography, or a biosensor assay.

58. The method of claim 56, wherein the contacting step is performed in cells and the detecting step comprises monitoring expression of a detectable gene product.

59. The method of claim 58, wherein the cells are yeast cells.

60. The method of claim 56, wherein the detecting step comprises screening a polypeptide expression library or a combinatorial peptide library.

61. The method of any one of claims 56 to 60, wherein the complex is formed by binding between at least one PDZ domain in the test amino acid sequence and a C-terminal sequence in the SYNGAP or fragment or derivative thereof having the following sequence: (T or S) XV; wherein X is an amino acid.

62. An amino acid sequence detected by any of the methods of claims 56 to 61.

63. A method for detecting excitatory synapses in a cell or group of cells, the method comprising contacting cells or a group of cells with the antibody or antigen-binding fragment of any one of claims 26 to 29 under conditions sufficient to detect the excitatory synapses in the cells or group of cells.

64. The method of claim 63, wherein the antibody or antigen-binding fragment is detectably-labeled or is capable of producing a detectable label.

65. The method of claim 64, wherein the antibody or antigen-binding fragment is labeled with at least one of a radionuclide; a protein tag; a chromophore; a fluorescent, chemiluminescent or phosphorescent molecule.

66. The method of claim 64, wherein the antibody or antigen-binding fragment thereof is labeled with at least one enzyme capable of producing a chromophore, a fluorescent, chemiluminescent or phosphorescent molecule.

67. A method of detecting a compound capable of modulating the Ras GTPase activity of SYNGAP, the method comprising the steps of:

a) transfecting a cells with a reporter gene construct capable of being modulated by Ras-Raf (MAP kinase),

b) transfecting the cells with a polynucleotide encoding any one of the SYNGAP sequences shown in SEQ ID Nos. 3, 6, or 9,

c) contacting the cells with a candidate compound; and

d) detecting the reporter gene construct as being indicative of the compound capable of modulating the RasGTPase activity of SYNGAP.

68. A library comprising any one of the polynucleotides of claims 1 to 20.


```

      10      20      30      40      50      60
      *      *      *      *      *      *
ATG TCC TAT GCG GCG TTC AGA GAT GTA CCG GGA CCG CCG ATG CAC CGA ACC CAA TAC GTT CAT
TAC AGG ATA CCG GCG AAG TCT CTA CAT GCG CCG GCG GGA TAC CTC GCG TCG GTT ATG CAA GTA
M S Y A P P R D V R G P P M H R T Q Y V H>

      70      80      90      100      110      120
      *      *      *      *      *      *
TCC CCG TAT GAC CCG CCG GGT TCG AAC CCG CCG TTC TCG ATC ATC TCT GCG AAC CAG CTG CTG
AGC GCG ATA CTG GCA GCG CCA ACC TTC GCG CCG AAG ACC TAG TAC AGA CCG TTG CTC GAC TAC
S P Y D R P G W N P R F C I I S G N Q L L>

      130      140      150      160      170      180
      *      *      *      *      *      *
ATG CTG CAT GAG CAT GAG ATA CAC CCG CTG CTG ATC CCG GAC CCG AGG AGC GAG TCC AGC CGA
TAC GAC CTA CTC CTA CTC TAT CTG GCG GAA GAC TAG GCG CTG CCG TCC TCG CTC AGG TCG GCT
M L D E D E I H P L I I R D R R S E S S R>

      190      200      210      220      230      240      250
      *      *      *      *      *      *      *
AAC AAA CTT CTG AGA CCG ACC GTC TCT GTG CCA GTG GAG CCG CCG CCG CAC CCG GAG CAT GAA
TTG TTT GAC GAC TCT CCG TCG CAG AGA CAG GGT CAG CTC CCG CCG GCG GTG CCG CTC GTA CTT
N K L L R R T V S V P V E G R P H G E E E>

      260      270      280      290      300      310
      *      *      *      *      *      *
TAC CAC TTG CCG CCG TCC AGG AGC AAC AGT GTC CCG CCG CCG AAA CAG TAC ACC ATG GAA CCG
ATC GCG AAC CCA GCG ACC TCC TCC TTC TCA CAG GCG CCG CCG TTT GTC ATG TCG TAC CTT CCG
Y H L G R S R R K S V P G G K Q Y S M E A>

      320      330      340      350      360      370
      *      *      *      *      *      *
GCG CCG GCT GCG CCG TTC CCG CCG TCG CAA GCG CTT CTG ACC CCG AGC CTA AAA ACC TCC ATC
CGC GCG CCA CCG GCG AAG GCG GCG AGC GTT TCG AAG CAC TCG CCG TCC GAT TTT TCG AGG TCG
A P A A P P R D S Q G P L S R R L K S S S>

      380      390      400      410      420      430      440
      *      *      *      *      *      *      *
AAA CCG ACA AAG TCA CAA CCG AAA CTT GAC CCG ACC AGC ACC TTT CCA CAG ATC CTG CTT CCG
TTT CCA TAT TTT AGT GAT GCG TTT GAA CTG GCG TCG TCG TCG AAA CCG GTC TCG GAC GGA GCG
K R T K S Q P K L D R T S S F R Q I L P R>

      450      460      470      480      490      500
      *      *      *      *      *      *
TTC CCA AGT GCT GAC CAT GAC CCG GCG CCG CTG ATG CAG AGT TTC AAG CAG TCT CAC TCC CAT
AAG GAT TCA CCA CTC GTA CTG GCG CCG CCG GAC TAC CTC TCG AAG TTC CTC AGA GTG AGG GTA
P R S A D H D R A R L M Q S F R E S H S H>

      510      520      530      540      550      560
      *      *      *      *      *      *
CAG TCC CTG CTG ACT CCG AGC AGT GAT GAT GAG GCG CTG GAC CTC AAC CTG CAT GAA GAC TCG
CTC AGG GAC GAC TCA GCG TCG TCA CCA CCA CTC CCG GAC CTC GAG TTG GAC CTA CTT CTG AGG
E S L L S P S S A A E A L E L N L D E D S>

      570      580      590      600      610      620      630
      *      *      *      *      *      *      *
ATT ATC AAC CCA GTA CAC AGC TCC AGT CTG GCG CAG GAG TTC TCG TTT GAG GTA ACA ACA TCG
TAA TAG TTC CCG CAT CTG TCG AGC TAG CAC CCG GTC CTC AAG ACC AAA CTC CAT TCT TCT ACC
I I K P V H S S I L G Q E P C F E V T T S>

      640      650      660      670      680      690
      *      *      *      *      *      *
TCT GCG ACA AAA TGT TTT GCG TCT CCG TCT CCA GAT GAA AGG GAC AAA TCG ATT GAG AAT CTA
AGA CCG TGT TTT ACA AAA CCG AGA GCG AGA CCG CCG CTT TCC CTC TTT ACC TAA CTC TTA CAT
S G T K C F A C R S A A E R D K W I E N L>

      700      710      720      730      740      750
      *      *      *      *      *      *
CAG ACC GCT GTG AAA CCG AAC AAG GAC AAC AGC CCG CCG GTA CAC AAG CTG CTG AAA CTA TCG
CTC TCC CCA CAC TTT CCG CTG TTC CCG TTC TCG GCG GCG CAT CTA TTG CAC GAC TTT GAT ACC
Q R A V K P N K D N S R R V D N V L K L W>

      760      770      780      790      800      810
      *      *      *      *      *      *
ATC ATA GAA GGT CCA GAG CTG CCG CCG AAG AAG CCA TAT TAC TCG GAC TTA TCG CTG GAC GAC
TAG TAT CTT CCA CCG CTC GAC CCG GCG TTC TTC GCT ATA ATG ACC CTC AAT ACC CAC CTG CTG
I I E A R W L P P R K K Y Y C H L C L D D>

      820      830      840      850      860      870      880
      *      *      *      *      *      *      *
ATC CTC TAT CCA CCG ACC ACT TCC AAG CCG CCG TCA GCG TCA GCA GAC ACT CTC TTT TGG GCG
TAC GAG ATA CCG CCG TCG TCA AGG TTC CCG CCG AGT CCG AGT CCG CTG TCA CAG AAA ACC CCG
M L Y A R T T S K P R S A S G D T V F W G>

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Figure 1A

820 900 910 920 930 940
 GAC CAC TTC GAC TTT AAC AAC CTC GGT GTC GGG GGG CTG GGG CTG CAT CTG TAC CGT GAC
 CTC GTG AAG CTC AAA TGG TGG CAC GGA CGA CAG GGG GGG GAC GAC GAC GTA GAC ATG GCA CTG
 E H F E F N N L P A V R A L R L H L Y R D>
 950 960 970 980 990 1000
 TCG GAC AAA AAG CGG AAC AAC CAC AAG GGA GGG TAC GTT GGT CTG CTG ACT GTT CCA GTG GCG
 AGC CTG TTT TTC GCG TTC TTC CTG TTT COT CCG ATC CAA CCG GAC CAC TGA CAA GGT CAC CGG
 S D K K R K K U K A G Y V G L V T V P V A>
 1010 1020 1030 1040 1050 1060 1070
 ACC CTG CTT GGG GCG CAC TTC ACA CAG CAG TGG TAC CCC GTG ACC CTG CCA ACA GGA AGT GGG
 TGG GAC CGA CCG GCG CTG AAG TGT CTC GTC ACC ATG GGG CAC TGG GAC GGT TGT CTT TCA CCC
 T L A G R H F W E Q W Y P V T L P T G S G>
 1080 1090 1100 1110 1120 1130
 GGC TGT GGT GGT ATG GCG TCG GGG GGA GGA GGG GGT TCA GCG GCG GCG TCA GCG GCG AAA GGG
 CCG AGA CCG CCA TAC CCG AGC GCG COT COT CCG CCG AGT CCG CCG CCG ACT CCG CCG TTT CCG
 G S G G M G S G G G G S G G S G S G S G K G>
 1140 1150 1160 1170 1180 1190
 AAA GGA GCG TGT CTT GGT CTG GGG CTC AAG GCG CTT TAC CAG ACA ATG AGT ATC CTG CCG ATG
 TTT CTT CCG ACA CCA CCA CAC CCG GAC TTC CCG CCA AUG CTC TGT TAC TCA TAG GAC CCG TAC
 K G C C P A V R L K A R Y Q T M S I L P M>
 1200 1210 1220 1230 1240 1250 1260
 GAG CTA TAT AAG GAG TTT GCA GAA TAT GTG ACC AAC CAC TAC CCG ATG CTC TGT GCG CTC CTC
 CTC GAT ATA TTC CTC AAA CCG CTT ATA CAC TGG TTC GTG AUG GCG TAC GAC ACA CCG CAC GAC
 E L Y K E F A E Y V T N H Y R M L C A V L>
 1270 1280 1290 1300 1310 1320
 GAG CCG CCG CTC AAT GTC AAG GCG AAG GAG CAG CTC GGT AGT GCA CTC GTT CAC ACT CTC CAA
 CTC GCG CCG GAG TTA CAG TTC CCG TTC CTC CTC CAG CCA TCA CCG GAC CAA GTG TAG GAC GTT
 E P A L N V K G K D E V A E A L V H I L Q>
 1330 1340 1350 1360 1370 1380
 AGC ACA GCG AAG CCG AAG GAC TTC CTT TCA GAC ATG GCG ATG TCA GAG CCA GAC CCG TTC ATG
 TCG TGT CCG TTC CCG TTC CTG AAC GAA AGT CTC TAC CCG TAC AGT CTC CAT CTC GCG AAG TAC
 S T G K A K D F L S D M A M S E V D R F M>
 1390 1400 1410 1420 1430 1440
 GAG CCG GAA GAC CTC AAT TTC CCG GAG AAC ACG CTC GCG ACT AAA CCG AAT GAA GAC TAT ATG
 CTC GCG CTT CTC GAG TAT AAG GCG CTC TTC TCG GAG CCG TGA TTT CCG TAT CTT CTC ATA TAC
 E R E H L I F R E N T L A T K A L E E Y M>
 1450 1460 1470 1480 1490 1500 1510
 AGA CTC ATT GCG CAG AAA TAC CTC AAG GAT GCG ATT GCG CAG TTC ATC CCG GGT CTC TAT GAA
 TGT GAC TAA CCG GTC TTT ATG GAG TTC CTA CCG TAA CCG CTC AAG TAG GCG CCA CAC AAT CTT
 R L I G Q K Y L K D A L G E F I H A L Y E>
 1520 1530 1540 1550 1560 1570
 TGT GAG GAG AAC TGT GAA GAA CAC CCG AAT AAG TCG ACA CCG TCG AGT CTG GCA GAG CAC CAG
 AGA CTC CTC TTG ACA CTT CAT CTC GGG TAG TTC ACG TGT CCG AGG TCA CAC CCG CTC CTC GTC
 S E R N C R V D P L K C T A S S L A E H Q>
 1580 1590 1600 1610 1620 1630
 GCG AAC CTC GCG ATG TCG TGT GAG TGG CCG CAG TCG AAG CTG CTC AAC TCG CAT TCG GTG TTC
 CCG TTC GAC CCG TAC ACG ACA CTC AAC CCG GAC ACG TTC CAC CAG TGG ACC GCA ACG CAC AAG
 A N L R M C C E L A L C K V V N S H C V E>
 1640 1650 1660 1670 1680 1690 1700
 CCG AGC GAG CTC AAC GAG GTG TTT GCA TCA TCG CCG CTG CCG TGT GCA GAG CCG GCG CCG GAG
 GCG TCG CTC GAC TTC CTC CAC AAA CTT ACT ACC GCG GAC GCG ACA CCG CTC GCG CCG CCG CTT
 P R E L K E V F A S W R L R C A F R G R E>
 1710 1720 1730 1740 1750 1760
 GAC AAT GGT GAC AGG CTC ATT AGC GCG TCG CTC TTT CTC CCG TTC CTC TCG CCG GCG GCG ATC ATG
 CAG TAA CCA CCG TGT CAC TAG TCG CCG AGC GAG AAG GAC GCG AAG CAG ACG GCG CCG TAG TAC
 D I A D R L I S A S L T L R F L C F A I M>

Figure 1B

1770 1780 1790 1800 1810 1820
 TCG CCC AGT CTG TTT GCA CTG ATG CAG GAG TAC CCA GAT GAG CAG ACC TCA CGA ACC CTC AGC
 AGC CCG TCA GAC AAA CCG GAC TAC GTC CTC ATG GGT CTA CTC CTC TGG AGT CCG TCG GAG TGG
 S P S L F C L M Q F Y P D E Q T S R T L T>

1830 1840 1850 1860 1870 1880 1890
 CTC ATC CCC AAG GTT ATC CAG AAC CTC GCC AAC TTT TCC AAG TTT ACC TCA AAG CAG GAC TTC
 GAC TAG CCG TTC CAA TAA GTC TTC GAC CCG TTG AAA AGG TTC AAA TGG AGT TTC CTC CTC AAG
 L I A K V T Q N L A N F S K F T S K E D F>

1900 1910 1920 1930 1940 1950
 CTC GCC TTC ATC AAC GAG TTT CTC GAG CCG GAG TCG GGT TCT ATG CAG CAA TTC TTC TAT GAG
 GAC CCG AAC TAC TTG CTC AAA GAC CTC GAC CTC ACC CCA AGA TAC GTC GTT AAG AAC ATA CTC
 L C F M N E F L P L E W G S M Q Q F L Y E>

1960 1970 1980 1990 2000 2010
 ATA TCC AAC CTC GAC ACA CTG ACC AAC AGC AGC AGT TTT GAG GGC TAC ATA GAC TTG CCC CCG
 TAT AGG TTG CAC CTG TGT GAC TCC TTG TCG TCG TCA AAA CTT CCG ATG TAT CCG AAC CCG GCG
 I S N L D T L T N S S S F N C Y I D L G R>

2020 2030 2040 2050 2060 2070
 GAG CTC TCC ACA CTT CAC GCC CTC CTC TCG GAG CTC CTC CCC CAG CTC AGC AAG GAA GCC CTC
 CTC GAG AGG TGT GAA CTC CCG GAC GAG ACC CTC CAC GAC GCG GTC GAG TCG TTC TTT CCG GAC
 E L S T L E A L L W E V L P Q L S K R A L>

2080 2090 2100 2110 2120 2130 2140
 CTG AAG CTC CCG CCG CTG CCG CCG CTC CTC AGC GAC ATC AGC ACA GCG CTC AAG AAC CCG AAC
 GAC TTC GAC CCG GCG GAC GCG CCG GAG GAG TCG CTC TAG TCG TGT CCG GAC TCG TTG CGA TTG
 L K L G P L P R L L S D L S T A L R N P N>

2150 2160 2170 2180 2190 2200
 ATC CAA AGG CAG CCG AGC CCG CAG AGC GAG CCG GGT CCG TCT CAG CCG ATG CTC CTC CCG CCG
 TAG GTT TCC CTC GCG TCG GCG CTC TCC CTC CCG CGA GCG AGA GTC GCG TAC CAC GAC GCG CCG
 I Q R Q P S R Q S E R A R S Q P M V L R G>

2210 2220 2230 2240 2250 2260
 CCC TCA GCC GAG ATG CAG GCG TAC ATG ATC CCG GAC CTC AAC AGC TCC ATC CAC CTT CAG TCC
 CCG AGT CCG CCG TAC CTC CCG AGC TAC TAC GCG CTC GAG TTG TCG AGG TAC CTC GAA GTC AGG
 P S A E M Q G V M M R D L N S S I D L Q E>

2270 2280 2290 2300 2310 2320 2330
 TTC ATG CCG CCA CCG CTC AAC AGC TCT AGC GAC ATG GGT CCG CTC CCG TCC CCA AAC AAC GAG
 AAG TAC CCA CCG CCG GAG TTT TCC AGA TAC CTC TAC CCA GCG GCG GCG AGG GGT TCC TTC CTC
 S M A R G I N S S M D M A R L P S P T K E>

2340 2350 2360 2370 2380 2390
 AAA CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
 TTT GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
 K P P P P P P G G G K D L P V V V S R P P L>

2400 2410 2420 2430 2440 2450
 GCG CCG TCC TCC CCA CCA TAC TCC AGC AGC AGC TCG GAC ATC ACA GAG CCG GAG CAG AAG ATG
 CCG GCG AGG AGG GGT CGT ATC ACC AGC TTG TCG AGC CTC TAA TGT CTC GCG CTC CTT TTC TAC
 A R S S P A Y C T S S S D I P E V E Q K M>

2460 2470 2480 2490 2500 2510 2520
 CTG AGT GTC AAC AAG ACT CTG TCC ATG CTC CAC CTC CAG GCG GAC GCG CCG CCG CCG CCG
 GAC TCA CAG TTG TTC TCA CAC AGG TAC GAC CTC GAC GTC CCG CTC CCG GGA CCG CCG CCG GAA
 L S V N R S V S M L D L Q G D G P G G R L>

2530 2540 2550 2560 2570 2580
 AAC AGC AGT AGT GTT TCC AAC CTC GCA CCG GTT GCG GAC CTC TTG CAC TCA AGC CAG CCG TCA
 TTT TCG TCA TCA CAA AGG TTG GAC CCG CCA CAA CCG CTC GAC AAC GTC AGT TCG GTC CGA AGT
 N S S S V S N L A A V G D L L H S S Q A S>

2590 2600 2610 2620 2630 2640
 CTC ACA GCA GCG TTC CCG TTG CCG CCG GCA CCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
 GAC TGT CCG CCG AAC CCG AAC GCG GCA CCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
 L T A A L G L R P A P A C R L S Q G S G S>

2650 2660 2670 2680 2690 2700

Figure 1C

TCC ATC ACA GCA GGC ATG CCC CTC AGC CAG ATC GGT GTC ACT AL AAT GGT GTC CCC CCC
 ACG TAG TGT CTT CCG CCC TAC GCG GAG TCG CTC TAC CCA CAG TGA TTC CTA CCA CAG GCG CCG
 S I T A A G M R L S Q M G V " T D G V P AS
 2710 2720 2730 2740 2750 2760 2770
 CAG CAA CTG CCG ATC COT CTT TCU TTC CAG AAC CTT CTA TTC CAT ATG GGT GCG CAT CCA CCA
 GTC GTT GAC GCG TAG GGA GAA ACC AAG GTC TTG CCA GAC AAG GTA TAC CCA CCG CTA CTT GGT
 Q Q L R I P L S F Q N P L P H M A A D G P
 2780 2790 2800 2810 2820 2830
 GCG CCC CCA GCA GGC CAT GGA XGG AGC AGT CCG CAT CTT CCA CTT TCG TCG CAT CAC CAC CAC
 CCC GCG GGT CGT CCG CTA CCG CCC TCG TCA CCG GTA CCA GGT CCA AGG ACG GHA GTC GTC GTC
 G P P A G H G C S S G H G P P S S H H H H
 2840 2850 2860 2870 2880 2890
 CAC CAC CAT CAC CAT CAC CCA GCG GGA GAA CCG CCA GCG CAC ACT TTT GCG CCG TTC CAT GCG
 GTC CTC GTA GTC GTA GTC CTT CCG CTT CTC GCG GGT CCG CTC TGA AAA CCG GCG AAG GTA CCG
 H H H H H R C G S P P G D T F A P P H G
 2900 2910 2920 2930 2940 2950 2960
 TAT AGC AAG ACC GAG GAC CTC TCT ACG CCG GTC CTT AAG CTT CCG GCG CCC TCG ATC CTT CAC
 ATA TCG TTC TCG CTT CTC GAG AGA TCT CCG CAG CCA TTC GCG GGA CCG CCG AGT TAG GAA GTC
 Y S K S E D L S T G V P K P P A A S I L H
 2970 2980 2990 3000 3010 3020
 AGC CAC AGC TAC ACT CAT GAG TTT GGA CCG TCT GGT ACT CAT TTT AGC CCG CCG CAC CTC TCA
 TCG GTC TCG ATG TCA CTA CXC AAA CTT GCG AGA CCA TCA CTA AAA TCG CCA CCG GTC GAG AGT
 S H S Y S D E F G P S G T D F T R R Q L S
 3030 3040 3050 3060 3070 3080
 CTT CCG GAC AAC CTA CAG CAC TTC CTC TCG CCG CCG CAG ATC ACC ACC GGT CCG CAG AGG CCA
 GAA GTC CCG ATG CAT GTC GTC TAC GAG ACC CCG CCG CTC TAG TCG TAG CTA GCG GTC TCG GGT
 L Q J N L Q H M L S P P Q I T I C P Q R P
 3090 3100 3110 3120 3130 3140 3150
 CTT CCG TCA GCG CCA CCA GCG ACC ACT GGT GAG CCG AGT GGT CCG GCG GGT CCG GCG CAG CCA
 CCA GCG ACT CCG CTT CTT CCG CCG TCA CCA CCG CCG TCA CCA CCG CCG CCG CCG GTC GGT
 A P E G P G G C S G C G S G G C G G G G G G Q P
 3160 3170 3180 3190 3200 3210
 CTT CCG TCG CAG AGG GCG AAA TCT CAG CAG TTG ACA CTC AGT GGT CCG CAG AAA CCG CCG CCG
 CCA CCG AAC GTC TCG CCG TTT AGA TTC CTC AAC TGT CAC TCA CCA CCG GTC TTT CAG GCG GCG
 P P L O R G K S Q C L T V S A A Q K P R P
 3220 3230 3240 3250 3260 3270
 TCC AGC GCG AAC CTA TTG CAG TCG CCG GAA CCA AGT TAT GGT CTT GTC CTT CCA CCG CCA CAG
 AGC TCG CCG TTC CAT AAC TTC AGG GCG CTT GGT TCA ATA CCA GGA CCG GCA CTT CCG CTT GTC
 S S G N L L Q S P E P S Y G P A R P R Q Q
 3280 3290 3300 3310 3320 3330
 AGC CTC AGC AAA CAG GCG AGC ATT GCG GCG AGC CCG GGT AGT GGT CCG GGA GCG CTT CCG GCG
 TCG GAG TCG TTT CTC CCG TCG TGA CCG CCG TCG CCG CCG TCG CCA CCG CCG CCG CCA CCG CCG
 S L S K E C S I G G S G G S G G G G G G G G
 3340 3350 3360 3370 3380 3390 3400
 CTC AAG CCG TCC ATC ACC AAG CAG CAT TTT CAG ACT CCA TCG ACC CCG AAG CCG AAG ATG CCG
 GAG TTC GCG AGG TAG TCG TTC CTC GTA AGG CTC TCA GGT AGG TCG GAC TTG GCG TCG TAC GCG
 L K P S I T K Q H S Q T P S T L N P T M P
 3410 3420 3430 3440 3450 3460
 GCG TCG GAG CCG ACT CTA GCG TCG GTC TCG AAT AGC CCG CAG CAG TCG CTT GAC ATC GAG AGT
 CCG AGC CTC GCG TCA CAT CCG ACT CAC AGG TTA TAC GGA GTC GAC AGG CCA CCG TAC CTC TCA
 A S E R T V A W V S N M P H L S A D I E S
 3470 3480 3490 3500 3510 3520
 CCA CAC ATT CAG CCG GAA GAG TAC AAG CTC AAG GAG TAC TCG AAG TCG ATG GAG GAG AGC CCA
 CTT GTC TAA CTC CCG CTT CTC ATG CTC GAC TTC CTC ATG AGC TTC AGG TAC CTC CTC TCG CTT
 A H I E R E E Y R L K E Y S K S M D E S R
 3530 3540 3550 3560 3570 3580 3590

Figure 1D

CTG GAC AGG GTG AAG GAG TAC GAG GAG GAG ATC CAC TCA CTC AAG GAA AGG CTA CAC ATG TCG
 GAC CTC TCC GAC TTC CTC ATG CTC CTC CTC TAG GTG AGT GAC TTC CTT TCC GAT CTG TAC AGG
 I. D R V K E Y E E E I H S L K R R L H M S>

3600 3610 3620 3630 3640 3650
 AAC CCG AAG CTG GAA GAG TAC GAG CCG AGG CTG CTG TCC CAG GAA GAG CAG ACC AGC AAG ATC
 TTG GCG TTC GAC CTT CTC ATG CTC CCC TCC GAC GAC ACC CTC CTT CTC CTC TCG TCG TTC TAG
 N K K I E E Y E R R L L S Q E E Q T S K L>

3660 3670 3680 3690 3700 3710
 CTG ATC CAG TAC CAA GCG CCG CTG GAG CAG AGC GAG AAG CCG TTC AGG CAG CAG CAG GTG GAG
 GAC TAC GTC ATC CTT CCG GCG GAC CTC CTC TCG CTC TTC CAG TCC GTC CTC CTC CAC CTC
 L M Q Y Q A R L E Q S E K H L R O Q Q V E>

3720 3730 3740 3750 3760 3770 3780
 AAG GAC TCC CAG ATC AAG AGC ATC ATT GCG AGG CTG ATG CTG CTC GAG GAG GAC CTC CCG CCG
 TTC CTG ACG GTC TAG TTC TCC TAG TAA CCG TCC GAC TAG GAC CAC CTC CTC CTC GAC CCG CCC
 K D S Q I K S I I G R L M L V E E S L R R>

3790 3800 3810 3820 3830 3840
 GAC CAC CCG GCG ATG GCT GAG CCG CTG CCG GAA CCG AAG AAC AGG CTG CTC CAC GCT CAG AGA
 CTG CTG GCG CCG TAC CGA TTC GCG GAG GGA CTC GCG TTC CTC TCC GAC GAG CTG CGA GTC TCT
 D H P A M A E P L P E P K K R L D D A Q R>

3850 3860 3870 3880 3890 3900
 CCG AGC TTC CCG CCG TCG CTC GAA GAA ACC CCG GTC TGA CCG TCG CCG CAC CTC CTT GGA ACG GCG
 CCG TCC AAG CCG GGA ACC CAC GTC GTC TCG ACC CAC ACT CCG ACC GGG GTC GAA CCG TCC CCG
 G S F U P W V Q Q T R V * R W P L L G T A>

3910 3920 3930 3940 3950 3960
 TGG CCG CCG CAC CTC CAC CCG CCG CAC CCG CCG TCG ACA TCA CAG ACA ACG GCG AGT TCC GGA
 ACC GCG GCG GTC CCG GTC CCG CCG GTC CCG CCG ACC TCG AGT CTC TCC TCC CCG TCA AGG CCG
 W P P Q P H P P H P C C R S Q R T A S S G>

3970 3980 3990 4000 4010 4020 4030
 ACA CCG CAC ACC ACT AGC CCA CCG AGC ACT ACA GAC CTC CTT CCG TGT GGA CCG TAC CCG GCG
 TGT GCG GTC TGG TGA TCG GGT CCG TCG TAG TGT CTC GAG GAA CCG ACA CCG GCG ATG GAG CCG
 T P Q T T S P P S L T D L L P C A P V P G>

4040 4050 4060 4070 4080 4090
 CCA CCG AGC CTC ACA GAC CAC CTT CCG AGT GGA CCG GAC CCG GGA ACA TCA CCA ACC ACC AGG
 GGT GCG TCC CAG TGT CTC GAG GAA GCG TCA CCG GCG CTC GGA CCG TGT AGT CCG TGG TGG TCC
 P P S V T D L L P S A P D P G T S P T T R>

4100 4110 4120 4130 4140 4150
 ACT GGA CCG CAC CAA GCG ACA GCG GGA TCG TGT CCG TTA ACG CCG CCG TCG CCG ACC CAT CTC
 TGA CCG GCA GTC CTT CCG TGT CCG CCG AAC AGA GCG AAT TCG GGA GGA ACC CCG TCG GTA GAC
 T G R H C G T A G L S P L T P P W G T H L>

4160 4170 4180 4190 4200 4210 4220
 TTA ACC CCA CTG CTC CAC TCC ACC ACG GAG AGT GCG ACG CTC ACC TCC CCG CAC ACC CCA GGA
 AGT TCG GGT GAC GAG GTA AGG TCC TCC CTC TCA CCG TGG GAG TCG ACC GGA GAG TGG CCG CCG
 S T P L L H S R R E S G T L S C P L T P G>

4230 4240 4250 4260 4270
 CAC CAC CTA CCG CAC ACA GAC CCG TTC ACT CCG GCG TCC TAC CCG CAT CCG
 CTC CTC CAT CCG CTC TGT CTC GCG AAG TGA GAC CCG ACG ACA GCG GGA GGA
 H H L P H T D P P T L G C Y P E P>

Figure 1E

Sequence Range: 1 to 4134

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      10      20      30      40      50      60
TAA GGC CCC CCA CUC CUA CUC GGT CAG GGG GGT CCG GTT CAG GTT CTT TGC CCC CTC CTT CCC
ATT CCG GCG GGT GUX CCT GCG CCA GTT CCG CCA GGC CAA GTT CAA GGA ACU GCG GAG GAA GTT
  C  P  P  P  R  P  R  Q  G  A  P  V  Q  V  P  C  P  L  L  P>

      70      80      90     100     110     120
ACC GGC AGC CTC TCC GUC CCC GGT GGT CTT CTT CTT GGT TTC CCG GGG AAT ACC ACT TGG GTC
TGC CCG TCG GAG AGG CCG CCG CCA CCA CAA CCA CCA AAG GCG CCG TTA TCG TGA ACC CAG
  T  A  S  L  S  A  A  A  A  L  P  A  A  F  R  G  N  T  T  W  U>

     130     140     150     160     170     180
GCT CCA CCA CCA AGA GTG TCC CCG GGG GGG AAA CAG TAC AGC ATG CAA GCG GCG CCG GGT GCG
CGA GAT CTT CTT TCT CAC AGG GCG CCC CCC GTT GTT ATG TCG TAC GTT CCG CCG GCG CCA GCG
  A  R  G  R  V  S  P  G  G  K  Q  Y  S  M  F  A  A  P  A  A>

    190     200     210     220     230     240     250
CCG TTC CCG CCC TCG CAA GCG TTC CTG AGC CCG AGG CTA AAA AGC ATT ATT AAA CCG ACA AAG
GGG AAG GCG GCG AGC GTT CCG TAC GAC TCG GCG TCG GAT TTT TCG AGG TAC TTT CCA TGT TTC
  P  F  R  P  S  Q  G  F  L  S  R  R  L  K  S  S  I  K  R  T  X>

     260     270     280     290     300     310
TCA CAA CCC AAA CTT CAC CCG ACC AGC AGC TTT CGA CAG ATC CTC CCG CCG TTC CCA AGT CCG
AGT GTT GCG TTT GAA CTG GCT TCG TCG TCG AAA GAT CTC TAG GAC GGA CCC AAG GGT TCA CCA
  S  Q  P  K  L  P  R  T  S  S  F  R  Q  I  L  P  R  F  R  S  A>

     320     330     340     350     360     370
GAC GAT GAC CCG GCG CCG CCG ATG CAG AGC TTC AAG TAC TCT CAC TCG CAT CAG TCG CTG CTC
CTG GTA CTG GCG GCG GCG GAC TAC GTC TCG AAG TTC CTC AGA GTG AGC GTA CTC ACC CAC GAC
  D  H  D  R  A  R  L  M  Q  S  F  K  E  S  H  S  H  E  S  L  L>

    380     390     400     410     420     430     440
AGT CCG ACC AGT GGT GGT GAG CCG CTC GAG CTC AAC CTG GAT GAA GAC TCG ATT ATC AAG CCA
TCA GCG TCG TCA CCA CCA CTC CCG GAC CTC GAG TCG GAC CCA GTT CTC AGG TAA TAG TTC GGT
  S  P  S  S  A  A  E  A  L  E  L  N  L  D  E  D  S  I  I  K  P>

     450     460     470     480     490     500
GTA CAC AGC TCG ATC CTG GCG CAG GAG TCG TCG TTT GAG GTA ACA ACA TCG TCT GCG ACA AAA
CAT GTG TCG ACC TAG GAC CCG CTC CTC AAG ACS AAA CTC CAT TGT TGT AGC AGA CCG TGT TTT
  V  H  S  S  T  L  G  Q  E  F  C  P  E  V  T  T  S  S  G  T  X>

     510     520     530     540     550     560
TCT TTT CCG TGT CCG TCT CCA GCG GAA ACC GAC AAA TCG ATT GAG AAT CTA CAG AGG GGT GTG
ACA AAA CCG ACA CCG AGA CCG CCG CTT TCG CTG TTT ACC TAA CTC TTA CAT GTT TCG CCA CAC
  C  F  A  C  R  S  A  A  E  R  D  K  W  I  E  N  L  Q  R  A  V>

     570     580     590     600     610     620     630
AAA CCG AAC AAG GAC AAC AGC CCG CCG GTA GAT AAC GTG CTG AAA CTA TCG ATC ATA GAA GGT
TTT GCG TCG TTC CTC TTG TCG GCG GCG CAT CCA TTC CAC GAC TTT GAT ACC TAG TAT CTT CCA
  K  P  N  K  D  N  S  R  R  V  D  N  V  L  K  L  W  I  I  E  A>

     640     650     660     670     680     690
CGA GAG CTG CCC CCC AAG AAG CCA TAT TAC TCG GAG TTA TCG CTC GAC GAC ATG CTC TAT GCA
GCT CTC GAC CCG GCG TCG TTC GGT ATA ATG AGG CTC AAT ACC GAC CTG CTG TAC GAG ATA CTT
  R  E  L  P  P  K  R  R  Y  Y  C  E  L  C  L  D  D  M  I  Y  A>

     700     710     720     730     740     750
CGG ACC ACT TCG AAG CCG CCG TCA CCG TCA CCA GAC ACT GTT TTT TGG GCG GAG CAC TTC GAG
GCC TCG TGA AGG TTC GCG GCG AGT CCG ACT CCG CTC TGA CAC AAA ACC CCG CTC GTG AAG CTC
  R  T  T  S  K  P  R  S  A  S  G  D  T  V  F  W  G  E  H  F  E>

     760     770     780     790     800     810
TTT AAC AAC CTG CTT GGT GTT CCG CCG CTG CCG CTC GAT CTC TAC CTT GAC TCG GAC AAA AAG
AAA TTT TTT GAC CCA CCA CAG CCG CCG GAC CCG GAC GTA GAC ATG CCA CTG AGC CTG TTT TTC
  F  N  N  L  F  A  V  R  A  L  R  L  H  L  Y  R  D  S  D  X  X>

    820     830     840     850     860     870     880
CGG AAG AAG GAC AAG GCA GCG TAC GTT CCG CTG GTG ACT GTT CCA GTG GCG ACC CTC CCG GCG
GCC TTC TTC CTC TTC CTT CCG AAT CAA CCG GAC CAC TTA CAA CCG CAC CCG TGG GAC CCA CCG
  R  K  K  D  K  A  G  Y  V  G  L  V  T  V  P  V  A  T  L  A  G>

```

Figure 2A

1770 1780 1790 1800 1810 1820
AAC CAG TTT CTG (TTT GAG TGG GGT TCT ATG CAG CAA TTC TTG TTA AG AEA TTT AAC CTG
TTC CTC AAA GAC C- GAC CTC ACC CCA AGA AAC GTC GTT AAG AAC ATA CTC TAT AGG TTG GAC
N E F L E L E W G S M Q Q F L Y E T S N L>

1830 1840 1850 1860 1870 1880 1890
CAC ACA CTG ACC AAC AGC AGC AGT TTT GAG GGC TAC AEA GAC TTG GGC CGC GAG CHU TUC AEA
CTG TGT GAC TGG TTG TCG TCG TCG TCG AAA CTC CGC ATG TAT CTG AAC CGC CGC CTC GAG AGG TGT
D T L T N S S S P E G Y I L L G R E L S T>

1900 1910 1920 1930 1940 1950
CTT CAC GGC CTG CTC TCG GAG GTG CTG CGC CAG CTC AGC AAG GAA GGC CTC CTG AAC CTG GGC
GAA GTG CGC CAC GAG AAT CTC CAC GAG GTC GTC GAG TCG TTC CTT CGC GAG GAC TTC GAC CGC
L H A L L W E V L P Q L S Y E A L L K L G>

1960 1970 1980 1990 2000 2010
CGC CTC GGC CGC CTC CTC AGC GAC ATC AGC ACA GGC CTG AGC AAC CTT AAC ATC CAA AGG CAG
GGC GAC GGC GGC GAG GAG TCG CTG TAG TCG TGT CGC GAC TCG TTG GAA TTG TAG GTT TCG GTC
P L P R L L S D I S T A L R N P N I Q R G>

2020 2030 2040 2050 2060 2070
CGC AGC GGC CAG AGC GAG GGC GGT GGC TTT CAG GGC ATG GTG CTG GGC GGC GGC GGC TCA GGC GAG
GGC TCG GGC GTC TTG CTC GGC CCA GGC AGA GAC GGC TAC CAC GAC GGC GGC GGC AGT CAG CTC
P S R Q S E R A R S Q P M V L R G P S A E>

2080 2090 2100 2110 2120 2130 2140
ATG CAG GGC TAC ATG ATG CGC CAC CTC AAC ACC TCG ATG CAG CTT CAG TTG TTC ATG GGT CCA
TAC GTC CGC ATG TAC TAC GGC CTC GAG TTG TCG AGG TAG CTC GAA GTC AGC AAG TAC CCA GGT
M Q G Y M M R D L N S S I D L Q S F M A R>

2150 2160 2170 2180 2190 2200
GGC CTC AAC AGC TTT ATG GAC ATG GGT GGC CTC GGC TCG CCA AGC AAC GAG AAA CCG CGC GGC
CTC CAG TTG TCG AGA TAC CAG TAC CCA GGC GAG CGC AGG GGT TCG TTG CTC TTT GGC GGC GGC
G L M E S M D M A R L P S P T K E K P P P>

2210 2220 2230 2240 2250 2260
CGC CTT GGC GGT GGC GGT AAA GAC CTG TTC TAC GTG ACC CGC CCA CCA CTG GGC CGC TCG TCG
CGC CCA CGC CCA CTC CCA TTT CTC GAC AAC AEA CAC TCG GUL GGT GGT GAC CGC CGC AGG AGG
P F P G G K D L F Y V S R P P L A R S S>

2270 2280 2290 2300 2310 2320 2330
CCA GCA TAC TCG AGG AGC AGC TCG GAC ATC ACA GAG CGC GAG CAG AAC ATG CTG AGT GTC AAC
GGT CTT ATC AGG TCG TCG TCG AGC CTC TAG TGT CTC GGC CTC GTC TTC TAC GAC TCA CAG TTC
P A Y C T S S S D I T E P Q K M L S V N>

2340 2350 2360 2370 2380 2390
AAG AGT GTG TCG ATG CTG GAC CTG CAG GGC GAC GAG GGT GGC GGC GGC CTT AAC AGC AGT AGT
TTC TCA CAC AGG TAC CAC CTG GAC CTC CGC CTG GGC CCA CCA CCA CCA GAA TTG TCG TCA TCA
K S V S M L D L Q G D C P G C R L N S S S>

2400 2410 2420 2430 2440 2450
GTT TCG AAC CTG CCA GGT GTT GGC GAC CTG TTG CAC TCA AAT CAG GGT TCA CTG ACA GCA GGC
CAA AGT TTG GAC CTT CCA CCA CCA CTC CAC AAC GTG AGT TCG GTC CCA AGT TAC TGT CTT CAG
V S N L A A V G D L L H S S Q A S L T A A>

2460 2470 2480 2490 2500 2510 2520
TTG GGC TTG GGC CTT GCA CTT GGC GGC GGC CTC TCG CAA GGC AGT GGC TGT TCG ATC ACA GCA
AAC GGC AAC CGC GGA GGT CCA GGC CGC GGC GAG AGG GTT CCG TCA CGC AGA AGG TAG TGT CTT
L G L R F A P A G R L S Q G S C S S I T A>

2530 2540 2550 2560 2570 2580
GGC GGC ATG GGC CTC ACC CAG ATG GGT GTC AGT AGC GAC GGT GTC CCG GGC CAC CAA CTG GGC
CGC CCG TAC CGC GAG TCG GGC TAC CCA GAC TCA TAC CTA CCA CAG GGC CGC GGC CTC GTT CAC GGC
A G M R L S Q M G V T T D G V P A Q Q L R>

2590 2600 2610 2620 2630 2640
ATC CTT CTT TCG TTG CAG AAC CTT CTC TTC CAT ATG GGT GGC GAT GGA CCA GGC CGC CCA GCA
TAG GGA GAA AGG AAG GTC TTG GGA GAG AAC GCA TAC CCA CGC CTA CTT CTT CCG GGC GGT CTT
I F L S F Q N P L F H M A A D C P G P P A>

2650 2660 2670 2680 2690 2700

Figure 2C

GGC CAT GGA GGG AGT GGC CAT GGT CUA GCT TCC TCC CAT CAC C CAC CAC CAC CAT CAC
 CCG GTA GGT GGG TCA GCG GTA CCA GGT GCA AGG AGG GTA GTG G JTG GTG GTG GTA VTG
 G H C G S S H H G P P S S H H L H H H H H H>
 2710 2720 2730 2740 2750 2760 2770
 CAT CAC CCA GGG GGA GAA GGC GCA GCG GAC ACT TTT GCG CCG TTC CAT GGC TAT AGC AAC AGC
 GTA GTG GGT GCG GCT CTT GCG GGT GCG GTG TCA AAA CCG GGC AAG GTT CCG ATA TCG TTC TCG
 H H R C G E P P G D T F A Y F H G Y S K S>
 2780 2790 2800 2810 2820 2830
 GAG GAC CTC TCT ACA GCG GTG GGT AAG CCG GGT GCG GCG TCC ATC CTT CAC AGC CAC AGC TAC
 CTC CTG GAG AGA TGT CCG CAC GGA TTC GCG GGA CCG CCG AGG TAG GAA GTG TCG GCG TCG ATG
 Z D L S T G V P K P P A A S I L Y S H S Y>
 2840 2850 2860 2870 2880 2890
 AGT GAT GAG TTT CCA CCG TCT GGT ACT GAT TTT ACC CTT CCG CAG CTC TCA CTT CAG GAC AAC
 TCA CTA CTC AAA CCG GCG AGA CCA TCA CCA AAA TCG CCA CCG GTC GAG AGT GAA TTC CCG TTC
 S D S F G P S G W D F T R R Q L S L Q D N>
 2900 2910 2920 2930 2940 2950 2960
 CTA CAG CAC ATG CAC TCT CCG CCG CAC ATC ACC ACC GGT CCG CAG ACC CCA GGT CCG TCA GCG
 GAT GTC GTG TAC GAG AGG CUC GCG GTC TAG TCG TAG CCA CCG GTC TCC GGT CCA CCG AGT CCG
 L Q H M L S P P Q I T I G P Q R P A P S Q>
 2970 2980 2990 3000 3010 3020
 CCA GGA GCG GCG AGT GGT GCG GCG AGT GGT GCG GCG CCG GCG CAG CCA CCG CCG TTC CAG
 GGT GGT CCG CCG TCA CCA CCG CCG TCA CCA CCG CCG CCG GTC GGT GGA CCG AAC CTC
 P Q G G H G G C S G G G G G G Q P P P L Q>
 3030 3040 3050 3060 3070 3080
 AGG GCG AAA TCT CAG CAG TTG ACA GTG ACT GGT GCG CAG AAA CCG CCG CCG TTC ACC GCG AAC
 TCC CCG TTT AGA GTC GTC AAC TGT CAC TCA CCA CCG GTC TTT GCG CCG GCG AGG TCG CCG TTC
 R C K S Q Q L W V S A A Q K P R F S S C N>
 3090 3100 3110 3120 3130 3140 3150
 CTA TTG CAG TCC CCG GAA CCA AGT TTT CCG CCG CCG CCG CCA CCG CCA CAG AGC CTC ACC AAA
 GAT AAC GTC AGG GAG CTT GGT TCA ATA CCA GGA ACC GCA GGT CCG GTC GTC TCG GAG TCG TTT
 L L O S P E P S Y G P A R P R Q Q S L S K>
 3160 3170 3180 3190 3200 3210
 GAG GCG AGC ATT GCG GCG AGG GCG GCG ACC GGT CCG GGA GCG GGT GCG GCG CTC AAG CCG TCC
 CTC CCG TCC TAA CCG CCG TCG CCG CCG TCC CCA CCG GGT CCG CCA CCG CCG GAG TTC CCG AGG
 E G S I G G S G G S G G G G C G G L K P S>
 3220 3230 3240 3250 3260 3270
 ATC ACT AAG CAG CAC TCC CAC ACT CCA TCC AAA CTT AAC TCC ACC AAG CCG CCG TCG GAG CCG
 TAC TCG TTC GTC GTA AGG CTC TGA GGT AAG TCG GAC TTC CCG TCC TAC CCG CCG ACC CTC GCG
 I W K Q H S Q W P S T L N P T M P A S E R>
 3280 3290 3300 3310 3320 3330
 ACT GTA GCG TCG GTG TCC AAT ATG CCG CAC CTG TCC CCG GAC ATC GAG AGT CCA TAC ACT GAG
 TGA CAT CCG ACC CAC AGG TTA TAC GGA GTG GTC AGC CCA CTG TAG CTC TCA CCG GTG TAA CTT
 W V A W V S N M P H L S A D I E S A H I R>
 3340 3350 3360 3370 3380 3390 3400
 CCG GAA GAG TAC AAG CTG AAG GAG TAC TCC AAG TCC ATG GAC GAG ACC CCA CTG GAC AGG GTC
 CCG CTT CCG ATG TTC GAC TTC CCG ATG ACC TTC AGG TAC CTC CTC TCG GGT GAC CTG TCC CAC
 R E E Y K L K E Y S K S M D E S R I D R V>
 3410 3420 3430 3440 3450 3460
 AAG CAC TAC GAG GAG GAG ATC CAC TCA CTG AAG GAA AGG CTA CAC ATG TCC AAC CCG AAG CTC
 TTC CTC ATG CTC CCG CTC TAC GTC AGT GAC TTC CTT TCC GAT GTG TAC AGG TTC CCG TTC GAC
 K E Y E E K I H S L K E R L H M S N R K L>
 3470 3480 3490 3500 3510 3520
 GAA GAG TAC GAG CCG ACC CTG CTG TCC CAG GAA GAG CAG ACC ACC AAG ATC CTC ATC CAG TAC
 CTT CTC ATG CTC GCG TCC GAC CAC AGG CTC CTT CTC GTC TCG TCG TTC TAG GAC TAC CTC ATC
 E E Y F R R L L S Q E E Q T S K I L M Q Y>
 3530 3540 3550 3560 3570 3580 3590

Figure 2D

CAA CCC CCC CTG GAG CAG AGC GAG AAG CGC TTG AGG CAG CAG CAG GTG GAG AAG GAC TCC CAG
 GTT CCG CCC GAC CTC GTC TCG CTC TTC GCG AAC TCG GTC GTC GTC CAG CTC TTC CTG AGG GTC
 Q A R L E Q S E K R L R Q Q Q V E K D S Q>

3600 3610 3620 3630 3640 3650
 AIC AAG AGC AIC AIC GGC AGG CIG AIG CTG GTC GAG GAG GAG CTG CCG CCG CAC CAC CCC CCC
 TAG TTC TCG TAG TAA CCG TCC GAC TAC GAC CAC CTC CTC CTC GAC CCG CCG CTC GTG GGG CCG
 T K S I I G R L M L V F E E L R R D H F A>

3660 3670 3680 3690 3700 3710
 ATG GGT GAG CCG CIG CCI GAA CCC AAG AAG AAG CTC CTC GAC GGT CAG ACA GGC AGC TTC CCC
 TAC CGA CTC GGC GAC GGA CTT GGG TTC TTC TCC GAC GAG CTC CGA GTC TCT CCC TCG AAG GGG
 M A E P L P E P K K K L H H A Q R G S F P>

3720 3730 3740 3750 3760 3770 3780
 CCT TCC CTC CAA CAA ACC CCG GTG TGA TCC TGG CCG CAC CTT GGA ACG CCG TGG CCC CCC CAG
 GGA ACC CAG GTT GTT TGG GCG CAC ACT CCC ACT CCG GTG GAA CCT TCC CCG AAT GCG GCG CTC
 P W V Q Q T R V * R W P H L G T A W P P Q>

3790 3800 3810 3820 3830 3840
 CCG CAG CCG CCG CAC CCG CCG TCC AGA TCA CAG AGA ACG GCG AGT TCC GGA ACA CCC CAG ACC
 GCG CTG GCG GCG GTG GCG CCG ACG TCC AAT GTG TCT TCC CCG TCA AGG CCT TGT GCG GTC TCG
 P H P P H P G C H S Q R T A S E G T P Q D>

3850 3860 3870 3880 3890 3900
 ACT AGC CCA CCC AGC AIC ACA GAC CTC CTT CCC TCT GCA CCG TAC CCG GGC CCA CCC AGC GTC
 TGA TGA GAT GAT TCG TAG TGT CTC CAG GAA GCG ACA CTT CCG AAT GCG CCG GGT GCG TCC CAG
 T B P P S I T D L L P C A P V P G P P S V>

3910 3920 3930 3940 3950 3960
 ACA GAC CTC CTT CCC AGT GCA CCG GAC CCT CCA ACA TCA CCA ACC ACC AGG ACT GGA CTT CTC
 TGT CTG GAG GAA GCG TCA CTT GCG CTG CCA CTT TCT ACT GGT TGG TGG TCC TGA CTT GCA GTC
 T D L L P S A P D P G T S P T T R T G R H>

3970 3980 3990 4000 4010 4020 4030
 CAA GCG ACA GCG GGA CTG TCT CCG TTA ACG CTT CTT TCC CCG ACC CAT CTG TCA ACC CCA CTG
 GTT CCG TGT CCG CCT AAC ACA GCG AAT TCG GGA GGA ACC CCG TGG GAA GAC AGT TCG GGT GAC
 Q G T A G L S P L T P P W G T H L S T P L>

4040 4050 4060 4070 4080 4090
 CTC CAT TCC ACC AGC CAC AGT GCG ACC CTC AGC TCG CTT CTC ACC CCA GAA CAC CAC CTA CCC
 GAC GTA ACC TCC TCC CTC TCA CCG TGG GAG TCG ACG GGA GAG TGG GGT CCT CTC CTC GAT GCG
 L H S R R E S G T L S C P L T P G H H L P>

4100 4110 4120 4130
 CAC ACA CAC CCG TTC ACT CIG GCG TCG TAT TCT CAT CTT
 CTG TGT CTG GCG AAG TGA GAC CCG ACG ACA GCG GTA GGA
 H T D P F T L C C Y P H P>

Figure 2E

Sequence Range: 1 to 3981

```

      10      20      30      40      50      60
TAC GGA GAG ACC GAG CTC GCG GAA GCA GCG GAT TTT GAA TTT GAT GCG GAG GAA ACC GAC CTC
ATC GCT CTC TGA CTC GAG GGG TTT GAT GGG GTA AAC GGT AAA GCA GGG GTC GTT GCG AAG GAC
  G  E  T  E  L  P  Q  A  P  R  F  F  F  A  P  Q  Q  G  F  L>

      70      80      90     100     110     120
AGC GCG ACC GTA AAA ACC TCG ACC AAA GGT ACA AAC TCA GAA GCG AAA GTC GAG GCG ACC AGC
TGG GCG TCG GAT TTT TCG AGG TAC GTT GCA TGT TTC AGT GTT GCG TTT GAA TTC GCG TCG TCG
  S  R  R  L  K  S  S  I  K  R  C  K  S  U  F  K  L  D  R  T  S>

     130     140     150     160     170     180
AGC TTT GGA GAG ACC GTC GCT GCG TTT GGA AGT GGT GAC GAT GAG GAG GCG GCG GTC ATG GAC
TCT AAA GCT CTC TAC GAG GCA GCG AAG GGT TCA GGA GCG GTA GTC GCG GCG GCG GAG TAC CTC
  S  F  E  Q  L  L  P  K  F  R  S  A  D  R  D  R  A  R  L  M  Q>

    190     200     210     220     230     240     250
AGC TTT AAG GAG TGT GAC TCG GAT GAG GCG GCG GTC AGT GCG ACC AGT GGT GGT GAG GCG CTC
TGG AAG TTT CTC AGA CTC ACG GTA CTC ACG GAC GAC TCA GCG TCG TCA GGA GGA TTC GCG GAC
  S  F  K  E  S  R  S  E  E  S  L  L  S  P  S  S  A  A  E  A  L>

     260     270     280     290     300     310
GAG CTC AAG CTC GAT GAA GAC TCG ATT ATT AAG GCA GTA GAC AGC TCG ATT ATT CTC GCG GAG GAG
CTC GAG TTT GAT GAA GTC CTC AAG TAA TAC TTT GGT CAT GTC TCG ACC GAG GAC GCG CTC CTC
  E  L  N  L  L  L  D  S  L  L  K  P  V  E  S  S  T  L  C  Q  E>

     320     330     340     350     360     370
TTT TCG TTT GAG GTC ACA ACA TCG CT GGG ACA AAA TGT TTT GCG TGT GCG TGT GGA ACC GAA
AAG ACC AAA CTC GAC TTT TTT ACC AGA GCG TGT TTT ACA AAA GCG ACA GCG AGA GGT GCG GGT
  C  C  F  E  L  L  S  E  G  C  K  C  F  A  C  R  G  A  A  E>

    380     390     400     410     420     430     440
AGC GAC AAA TCG ATT GAC AAT GTC GAG ACG GGT GTC AAA GCG AAC AAG GAC AAC AGC GTC GCG
TCC CTC TTT ACC TAA CTC TTA GAG CTC TCG GGA TAC TTT GCG TTT TTT TTT TTT TTT TTT TTT
  L  D  K  W  T  E  N  L  G  R  A  V  K  T  N  K  D  N  S  R  T>

     450     460     470     480     490     500
GTA GAT AAG CTC CTC AAA GTA TCG ATT ATA GAA GGT GCA GAG CTC GCG ACC AAC AAG GGA TAT
GAT GAT TCG GAC GAC TTT GAT ACC TAC TAC CTC GGA GGT GTC GAG GCG GCG GCG TTT TTT TTT TTT
  V  D  W  V  L  K  L  L  L  L  E  A  R  E  L  P  F  K  V  R  Y>

     510     520     530     540     550     560
TAC TCG GAG TTA TCG CTC GAT GAC ATT GTC TAT GCA GCG ACC ACT TCG AAG GCG CTC TCA GCG
ATG ACC CTC AAC ACC GAC CTC CTC TAC GAG ATA GGT GCG TCG TGA AGG TTT GCG GCG AGT GCG
  Y  G  E  L  C  L  D  N  X  L  V  A  R  T  T  S  K  F  R  S  A>

    570     580     590     600     610     620     630
TCA GGA GAC ACT GTC TTT TCG GCG GAG GAC TTT GAG TTT AAC AAC CTC GGT GGT GTC GCG GCG
ACT GGT GTC TGA GAG GAA ACC GCG CTC CTC AAG CTC AAA TTT TTT TTT TTT TTT TTT TTT
  S  G  D  T  V  F  W  G  S  H  E  E  F  N  V  L  S  A  V  R  A>

     640     650     660     670     680     690
CTG GCG CTC CAT CTC TAC GAT GAC TCG GAC TCG AAA AAG GCG AAG AAG GAC AAG GCA ACC TAC TTT
GAG GCG GAG TTA GAC AAG GAA TTT GAT ACC TAC TAC CTC GGA GGT GTC GAG GCG GCG GCG TTT TTT
  L  R  L  L  L  Y  R  D  S  D  K  K  R  K  K  D  K  A  G  V  Y>

     700     710     720     730     740     750
GCG CTC GTC ACT GTC CCA CTC GCG ACT CTC GGT GCG GCG GAC TTC ACA GAG GAG TTT TAC GCG
GCG GTC CAT TTA GAA GGT GAC GCG TCG GAC GGA GCG GCG GTC AAG TGT CTC CTC ACC ATG GCG
  G  L  V  T  V  P  V  A  T  L  A  G  R  H  F  T  E  Q  W  Y  E>

     760     770     780     790     800     810
GTC ACC CTC GCA ACA GCA ACC GCG GCG TGT GCG GGT ATG GCG TCG GCG GGA GGA GAC GCG TCA
CAC TCG GAC GGT GGT GGT TCA GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
  V  T  L  P  T  G  S  G  G  S  G  G  M  G  S  G  G  G  G  G  G  S>

    820     830     840     850     860     870     880
GCG GCG GCG TCA GCG GCG AAA GCG AAA GGA GCG TGT GGT GGT GTC GCG CTC AAG GCG GGT TAC
GCG GCG GCG ACC GCG GCG TTT GCG TTT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT
  G  C  C  S  G  R  X  G  K  G  G  C  P  A  V  R  L  K  A  R  Y>

```

Figure 3A

890 900 910 920 930 940
 CAG ACA ATC AGT ATT GTG GTC AAG GAG GGA GTC TTT GCA GAA TAT GTC ACC AAC CAC
 CTC GGT GAC TCA GAG GAG GGG GAG GTC GAT ATA TTC CTC AAA GGT TTT ATA GAG TGG TTG GTG
 Q - A G I L P H E L V K E F A S Z V T N H>
 950 960 970 980 990 1000
 TAC GGC AGT GTG GGT GCG GTG GTG GAG GCG GCG GTC AAT TTC AAG GCG AAG GAG GAG GTT GGT
 ATG GCG TAC GAG ACA GTG GAG GAG GTC GCG GAG GAG TTA GAG TTT GCG TTC GTC GTC GAG CGA
 Y R A L C A V L H P A L N V K G K S E V A>
 1010 1020 1030 1040 1050 1060 1070
 AAT GCA GTG GTT GAC ATT GTG GAA AGC ACA GCG AAG GCG AAG GAC TTC GTT GCA GAC ATT GCG
 TCA GGT GAG GAA GTG TAG GAG GGT GAT TGT GAG GTC GCG GAG GAG TTT AAG GAG GTC GAC CGA
 S A L V H I L Q H T G K A K D F L S D M K A>
 1080 1090 1100 1110 1120 1130 1140
 ATC GCA GAG GTA GAC GCG GTC ATT TAC GCG GAA GAC GTC ACA TTC GCG GAG AAC AGC GTG GCG
 TAC AGT GGT GAT GTG GCG AAG TAC GTC GCG GTC GTC GAT TAT AAG GCG GTC TTC TCG GAG GCG
 M S E V U A F H E R E Y L I F R E V L E A>
 1150 1160 1170 1180 1190
 AAT AAA GCG AAT GAA GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
 TCA TTT GCG TAT GTT GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
 I K A I E V Y H R L I G Q K F L K D A I G>
 1200 1210 1220 1230 1240 1250 1260
 GAG TTC ATT GGT GAT GCG GTC TAT GAA TAT GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
 CTC AAC GAG GCG GCA GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
 E F I R A L V E S E Y C F V D P I K C Q>
 1270 1280 1290 1300 1310 1320
 GCG TTC AGT GTG GCA GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
 CAG AAG TCA GAG GGT GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
 A S S L A E H Q A V L Y M C C E L A L C K>
 1330 1340 1350 1360 1370 1380
 CTC GTC AAC TCC GAT TCC GTC TTC GCG AAG GAG GTC AAG GAG GTC TTT GCA TCA TCG GCG GTC
 CAC GAT TCG ACC GCA AGG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
 V V N S H C V F P R E L K E Y F A S W R L>
 1390 1400 1410 1420 1430 1440
 GCG TGT TCA GAG GCG GCG GCG GCG GCG GAG ATT GGT GAG AAG GTC AGC AGC GCG TCG GTT TTC CTC
 GCG ACA GGT TTC GCG GCG GCG GCG GCG GCG GCG TAT TCA GCG TCG GAG TAG TCG GCG ACC GAG AAG GAG
 R C A L R G R E D I A D R L I S A S E F L>
 1450 1460 1470 1480 1490 1500 1510
 GCG TTC CTC TCC GCG GCG ATT ATT TCC GCG AGT CTC TTT GCA GTC ATG GAG GAG TAC GCA GAG
 GCG AAC GAG ACG GCG GCG TAG TAC ACG GCG TTA GAG AAA GCG GAG TAC GTC CTC ATG GGT GTC
 R P L C F A I M S P S L F G L M Q E Y P D>
 1520 1530 1540 1550 1560 1570
 GAG GAG ACC TCA GCA ACC CTC ACC CTC ACC GCG AAG GTT ATT CAC AAC CTC GCG AAC TTT TTC
 CTC GTC TCG AGT GGT TCG GAG TCG GAG TAC GCG TTT GAA TAG GTC TTC GAG GCG TTC AAA ACC
 E Q T S R D L I L A K V F O M L A N F S>
 1580 1590 1600 1610 1620 1630
 AAG TTT ACC TCA AAG GAG GAG TTC CTC GCG TTC TCC ACC AAG GAG TTT CTC GAG GAG TCG GGT
 TTT AAA GCG AGT TTC CTC CTC GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
 K F T S K S D S L G P H N E P L E L E W Q>
 1640 1650 1660 1670 1680 1690 1700
 TCT ATG GAG GCA TTC TTT TAT GAG ACA TCC AAC CTC GAG GCA GCA CTC ACC AAC ACC ACC AGT TTC
 AAA TAC GTC GTT AAG AAC ACA CTC TAT AAG TCG GAG CTC TGT GAG TCG TTC TCG TCG TCA AAA
 S H Q Q F L Y E I S N L D T L T N S S S P>
 1710 1720 1730 1740 1750 1760
 GCG GCG TAC ATA GAG TCG GCG GCG GAG GTC TCC ACA CTC CAC GCG CTC CTC TCG GAG GTC CTC
 CTC GCG ATG TAT CTC AAC GGT GCG CTC GAG AAG TTT GAA GTC GCG GAG GAG ACC CTC GAG GAG
 E G Y I Q L G R E A S T L H A L L W E V L>

Figure 3B

[illegible]

THE UNIVERSITY OF CHICAGO

THE **WORLD'S** **LARGEST** **BOOKSTORE**

Figure 3D

AAG AGG GGG GGC GAC GGT GAG AGA AGC AGT GTC GAA GAA ACC GCG GTC GGA
 TTC TCC GAC GAC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
 K R L L A Q R G E F P P W V Q Q F S V S
 3600 3610 3620 3630 3640 3650
 GCG TCG GCG GAC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
 GCG ACC GCG GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
 R W P L G C A W P P Q P H P P H P G C R
 3660 3670 3680 3690 3700 3710
 TCA CAG ACA AGG GCG AGT TCG GGA ACA GCG GAC AGT AGT AGC CCA GCG AGC ATC ACA GAC GTC
 AGT GTC TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG
 S Q R T A S S G T P Q T T S P P S L T D L
 3720 3730 3740 3750 3760 3770 3780
 CTT CCG TGT GCA GCG GAC GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
 GAA GCG ACA GTC GCG ACC GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
 L P C A E Y S G P P S V T S L L L P S A P D
 3790 3800 3810 3820 3830 3840
 CCG GCA ACA TCA GCA ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC
 GCA CCG TCG AGT GGT TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG
 P S T S P T T R T G A H Q G C A G L S P L
 3850 3860 3870 3880 3890 3900
 ACC CCG CCG TCG GCG ACC GAT CCG TCA ACC GCA CCG CCG GAC TCG AGG AGG GAG AGT CCG ACC
 TCG GCA GCA ACC TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG
 C P P W G T H L S T P L L H S H R S S G P
 3910 3920 3930 3940 3950 3960
 CTC AGG TCG ACC CCG ACC ACC GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA
 GAG TCG ACC GCA GCG TCG GGT CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
 L S C P L T P Q H H L P H T D P T T L S C
 3970 3980
 TAT CCG GAC CCG
 ATA GCG GTA GGA
 Y P H P

Figure 3E

1	MQSFKEHSHESLLSPSSAAEALNLDSDSIKPVHSSILGQEFCEVTTSSGKCFAC	60
61	RSAAERDKWIENLQRAVKPNKCNRRVDNVLKLWITEARELPKKRYCYELCLDDMLYAR	120
121	TTSKPRSASGDTVFWGEHFEFNNLPAVRALRLHLVYRSDKRRKKDKAGYVGLVTVFVATL	180
181	AGRHFTEQWYFVTLPTGSGGSGGMSGGGGGSGGGSGGKGGC?AVRLKARYQTMSILP	240
241	MELYKEFAEYVTNHYRMLCAVLEPALNVKKEEVASALVHILQSTGKAKDFLSDMAMSEV	300
301	DRFMEREHLIFRENTLATKAIEEYMRLIGQKYLKDAIGEFIRALYESEENCEVDPIKCTA	360
361	SSLAEHQANLRMCCELALCKVVNSECVFPRELKEVFASWRLCAERGREDIADRLISASL	420
421	FLRFLCPAIMSPSLFGLMQEYPDEQTSRTLTTLIAKVIQNLANSKFTSKEDFLGFMNEFL	480
481	ELEWGSQQFLYEISNLDLTNSSSFEQYIDLGRSLTLHALLWEVLPQLSKEALLKLG?	540
541	LPRLSDISTALRNPNIOROPSRQSEPARSQPMVLRGSAEMQGYMMDLNSSIDLQSF	600
601	ARGLNSSMDMARLPSPTKEKPPPPPPGGGKDLFYVSRPPARSSPAYCTSSSDITEPEQK	660
661	MLSVNKSVMMLDLQGDGPGGRLNSSSVSNLAAGVDDLHSSQASLTAAALGLRPAPAGRLSQ	720
721	GSGSSITAAGMRLSQMGVTTDGVPAQQLRIPLSFQNPFLHMAADGPGPPAGHGGSSGHG?	780
781	PSSHHHHHHHHHRGGEPPGDTFAPFHGYSKSEDLSTGVPKPPAASILHSHSYSDEFGPS	840
841	GTDFTRRQLSLQDNLQHMLSPQITIGPQRPAPSGPGGGSGGGSGGGGGQPPPLQRGKS	900
901	QQLTVSAAQKPRPSSGNLLQSPEPSYGPAPPPCOSLSKEGSISSSGSGGGGGGLKPSI	960
961	TKQHSQTPSTLNPTMPASERTVAWVSNMPLSADIESAHIEREEYKLKEYSKSMDESRLD	1020
1021	RVKEYEEIHSLSKERLHMSNRKLEEFYERRLLSQEEQTSKILMQYQARLEQSEKRLRQQQV	1080
1081	EKDSQIKSIIGRLMLVEEELRRDHPAMAEPLPEPKKRLDAQRGSFP9WVQQTRV	1135

Figure 4A

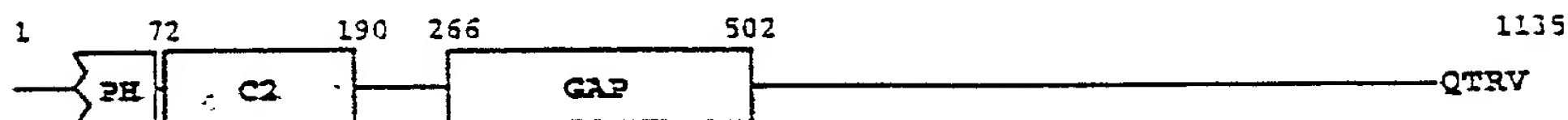


Figure 4B

+ * +			
GAPSYN	286	GKAKDFLSDMAMSEVDRFMERE-HLIFRENTLATKAIEEYMRLIGQKYLKDAIGEFIRALYE	347
rn GAP	755	KLESLLCTLNDREIS--MEDEATTLPFRATTLASTLMEQYMKATATQFVHALKDSILKIME	815
hs NF1	1251	HLLYQLLWNMFKEVE--LADSMQTLFRGNLSLASKIMTFCFKVYGATYLOKLL-DPLLRIVI	1311
GAPSYN	348	SEE-----NCEVDPIKCTAS-SLAHQANLRMCCELALCKVVNSHCVFPRELKEVFASWRLR	403
rn GAP	816	SKQ-----SCELSPSKLEKNEDVNTNLHLLSILSELVEKIFMASEILPPTLRYIYGCLQKS	872
hs NF1	1312	TSSDWQHVSFEVDPTRLEPSESLEENQRNLLQMTKFFHAISSSSSEFP9QLRSVCHCLYQV	1372
++ +			
GAPSYN	404	CAER--GREDIADRLISASLFLRFLCPAIMSPSLFGLMQEYPDEQTSRTLTTLIAKVIQNLAN	463
rn GAP	873	VQHKWPTNNTMRTRVVSGFVFLRLICPAILNPRMFNIISDSPSPLAARTLTLVAKSVQNLAN	934
hs NF1	1373	VSQRFPPQNSIG---AVGSAMFLRFINPAIVSPYEAGILDKKPPPRIERGLKLMKILQSIAN	1430
++			
GAPSYN	464	FSKFTSKEDFLGFMNEFLELE-WGSMQQFLYEISNLDLT	502
rn GAP	935	LVEFGAKEPYMEGVNPFIXSN-KHRMIMFLDELGNVPELP	973
hs NF1	1431	HVLF-TKEEHMRPFNDFVKSNEFDAARRFLDIASDCPTSD	1469

Figure 4C

Pleckstrin Homology(PH) Domain Alignment

SynGAP-a	162	FKESHSHESL	LSPSSAA...EAL	NLD.....EDSI	IK	19
Hs p120	471	FYKNIVKKG	YLLKKGKG...	..KRWK	NLYF	ILEGSDAQLI	YFESEK	RATK 51
Dm GAP1	759	PVLLKEGEGL	MTKYPTSRKR	FGRQFKQRHF	RLTT..HSL	YAKSK..GKQ		80
Hs Plec	1	MEPKRIREGY	LVKKGSV...	.FNTWKPMWV	VLL	..DGIE	FYKKK.SDNS	4

SynGAP-a	193	P.....VHSSIL	GQ.EFCFEVT	TSSG	TKC...	...FACRSAAE	22
Hs p120	516	PKGLIDLSVC	SVYVVHDSLF	GR.PNCFQIV	VQHFSEEHYI	FYFAGETPEQ		56
Dm GAP1	805	PICDIPLQEI	ASVEQLKDKS	FKMQNCFKIV	HND	RS.....	LIVQTTNCVE	84
Hs Plec	45	PKGMIPLKGS	TLTSPCQDFG	KR.MFVFKIT	TTKQQ.....	D	HFFQAAFLER	8

SynGAP-a	224	RDKWIENLQR	AVKPNKDNSR	243
Hs p120	565	AEDWMKGLQA	FCNLRKSSPG	584
Dm GAP1	850	EREWFDLLHK	ICLMNSIRMQ	869
Hs Plec	89	RDWVRDINK	AIKCIEGGQK	108

Figure 5

C2 Domain Alignment

SynGAP-a	245	..VDNVLKLW	..IIEARELPPKKRY.	YCELCL....	DDMLYARTTS	281
Hs p120	592	..QVSSLVLH	..IEEAHKLP	...VKHFTNP	YCNLYL....	NSVQVAKTH.	629
Rn Syt II	151	DYDFQANQLT	VGVLQAAELP	ALDMGGTSDP	YVKVFLLPD.	.KKKKYETKV	198
Bt Rab 3A	411	LYDQDNSSLH	CTIHKAKGLK	PMDSNGLADP	YVKLHLLPGA	SKSNKLRTKT	460

SynGAP-a	282	KPRSASGDTV	FWGEMHEFNN	LP.AVRALRL	HLYRD.SDKK	RKKDKAGYVG	329
Hs p120	630	...AREQONP	VWSEEFVDD	LPPDINRFEI	TL...SNKT	.KKSKDPDIL	671
Rn Syt II	199	...HRKTLNP	AFNETFTFKV	..PYQELGGK	TLVMAIYDFD	RF.SKHDIIG	242
Bt Rab 3A	461	...LRNTRNP	IWNETLVYHG	I.TDEDMQRK	TLRISVCDED	KF.GHNEFIG	505

SynGAP-a	330	LVTVPVAT..LAGRHFTEQ	WY.PVT.LPT	354
Hs p120	672	FMRCQLSR..LQKGHATDE	WFLSSSHIPL	698
Rn Syt II	243	EVKVPMNTVDL..GQPIEE	WRDLQG..G.	266
Bt Rab 3A	506	ETRFSLKCLK	PNQRK.NFNI	CL..ERVIPM	KRAGTTGSAR	542

Figure 6

Figure 7A

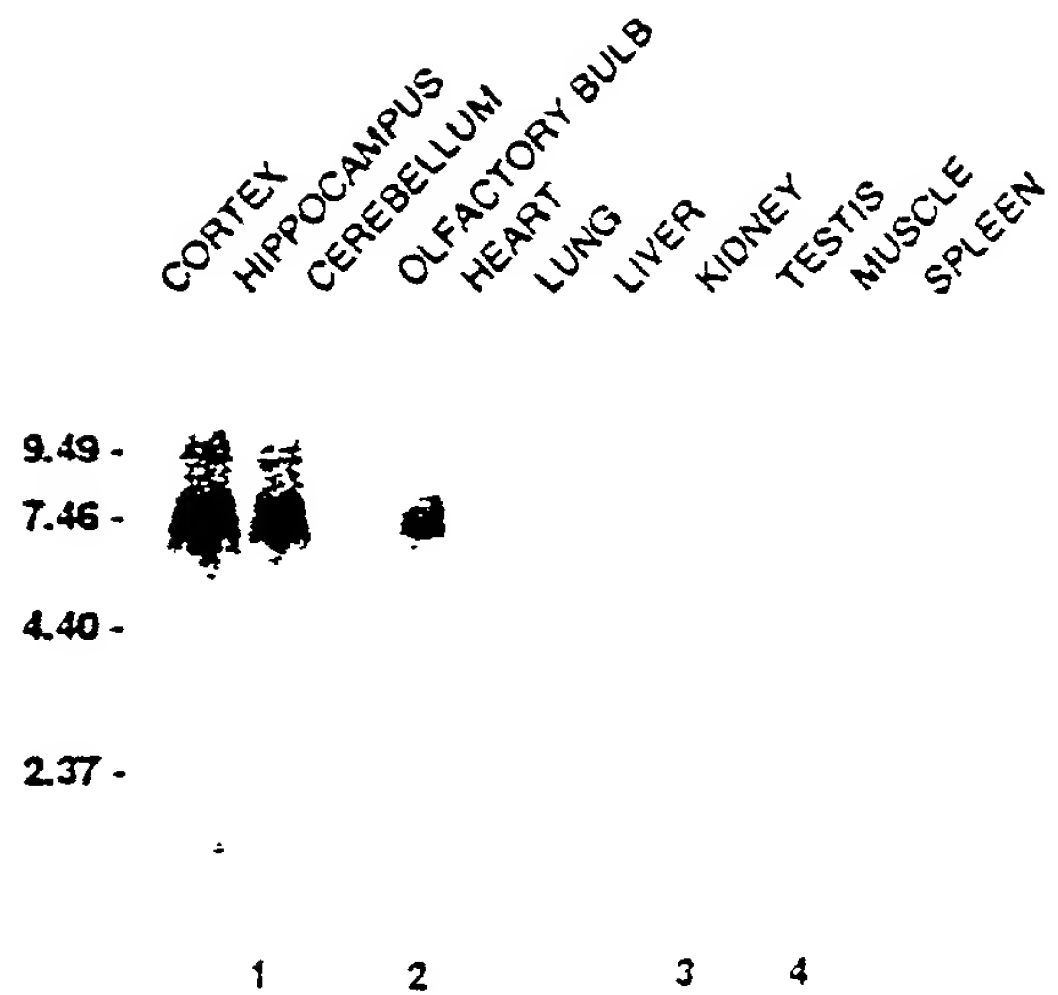


Figure 7B



Figure 7C

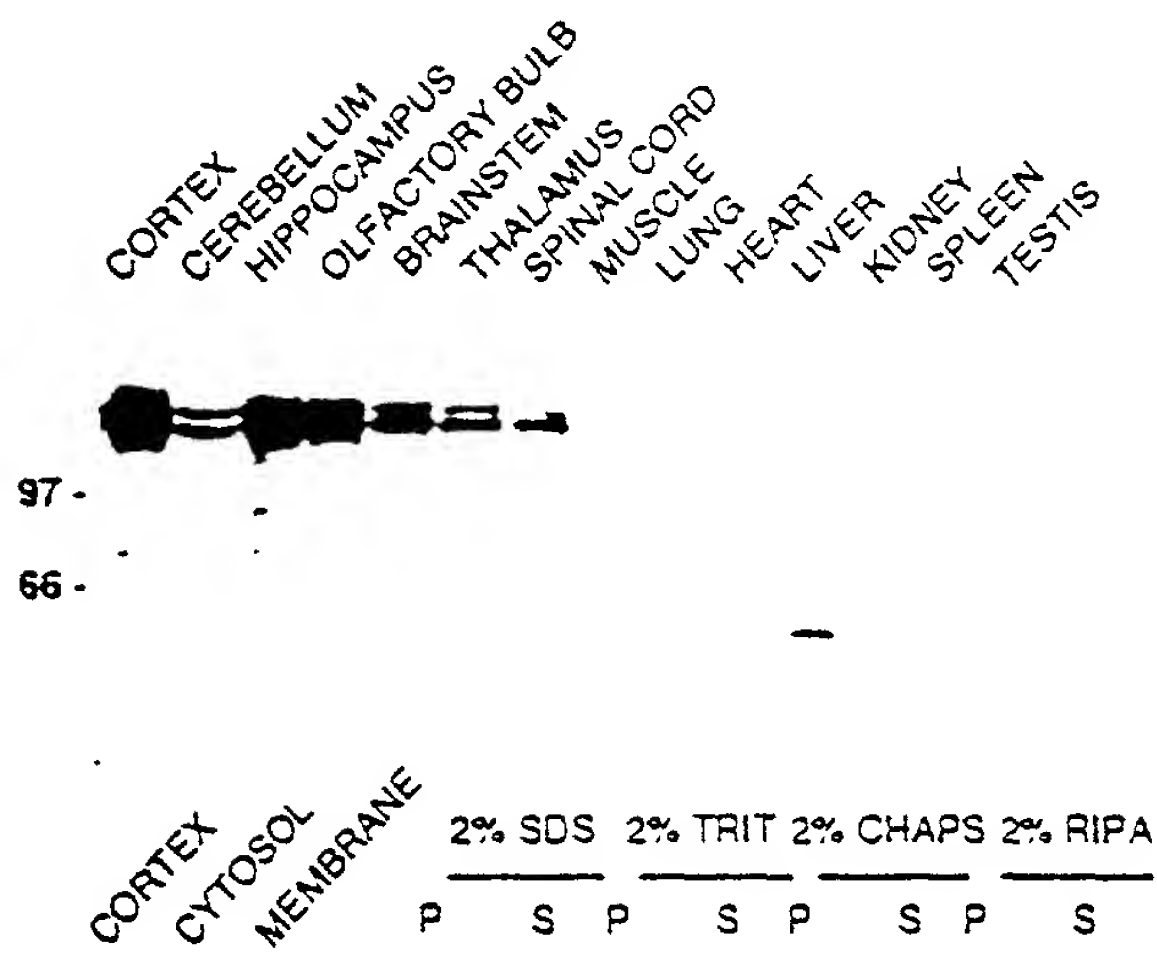
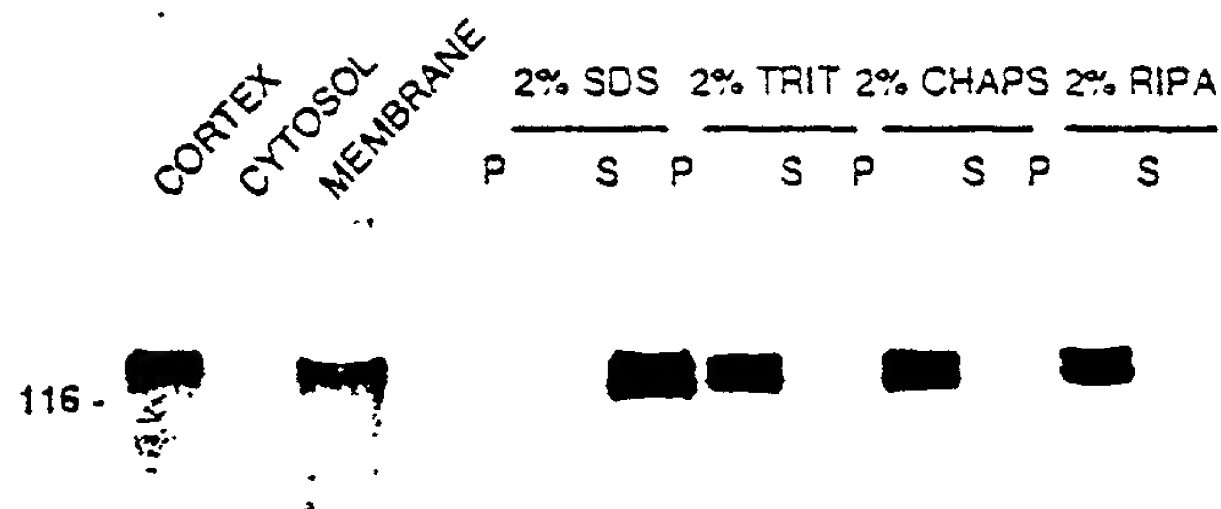


Figure 7D



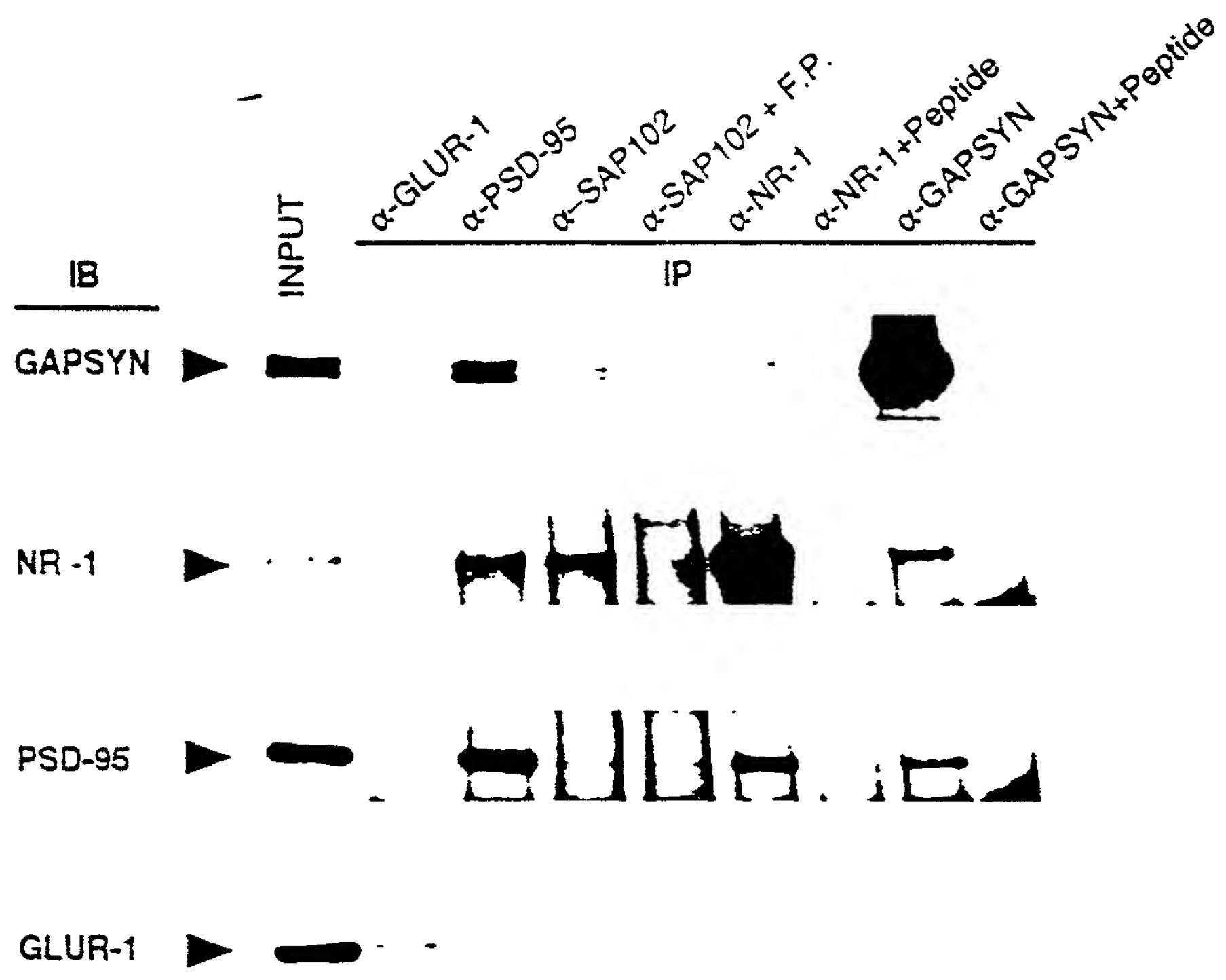


Figure 8

GAPSYN



Figure 9A

GAPSYN



Figure 9B

SYNAPTOPHYSIN



Figure 9C

OVERLAP



Figure 9D

GAPSYN (BLOCK)



Figure 9E

SYNAPTOPHYSIN



Figure 9F

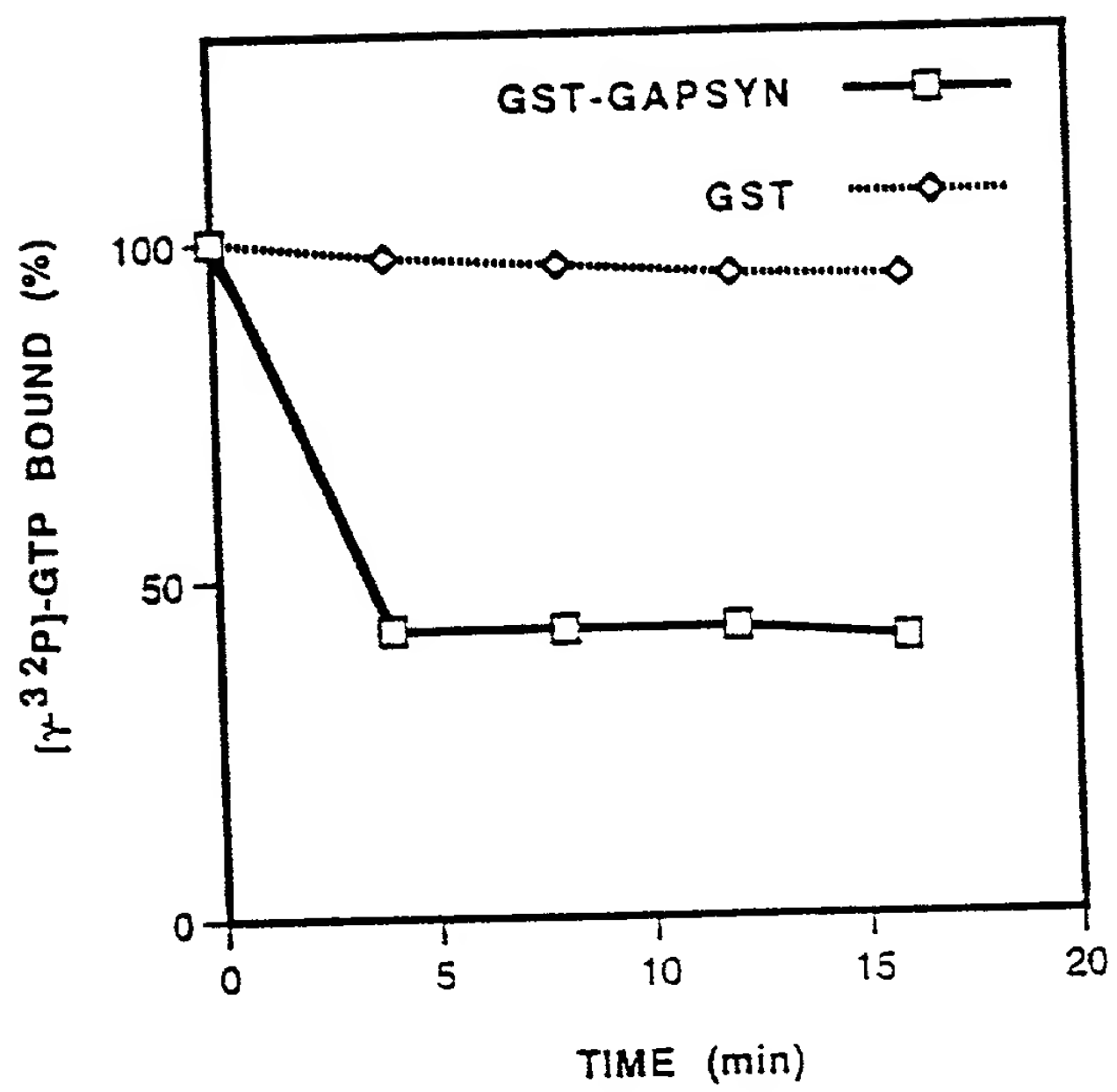


Figure 11